Cover image
Derivative work of a 3D reconstruction of z stacks showing DNA and β-catenin staining in a high-grade serous ovarian cancer tissue. The image was collected using the Leica TCS SP5 MP microscope. In this image, the DNA is pseudo-coloured in rainbow and β-catenin is red. Original image provided by Gayathri Chandrasekaran (Gergely laboratory).
Lineage tracing from small intestinal stem cells. Taken seven days post induction of tdTomato reporter (red), Phalloidin to label actin (green) and DAPI to stain nuclei (blue). Image provided by Anna Nicholson (Winton laboratory).
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The Cancer Research UK Cambridge Institute

The Institute’s location on the Cambridge Biomedical Campus facilitates collaborations with Addenbrooke’s Hospital and other Institutes and University of Cambridge Departments on the site.
This year, the sixth since the Institute opened, has seen both a consolidation of our scientific themes, and the preparations for important change.

The Institute has maintained its output of high impact papers, despite the loss of two outstanding senior group leaders: Fiona Watt to head a new Stem Cell Institute at King’s College London, and Dave Tuveson to the Cold Spring Harbor Laboratory.

We have had our regular programme of meetings, as well as some notable events and notable individual successes. In April, we welcomed Dr Robert (Bob) Weinberg (Whitehead Institute of Harvard and MIT) and his wife, Amy, for a one-month sabbatical. Dr Weinberg spent time at the Sanger Centre, the Gurdon Institute and the MRC-LMB as well as the CI. Highlights included lively structured discussions aimed at students and postdocs on topics including ‘cancer stem cells’ and ‘tumour heterogeneity’. Our thanks to Bob and Amy for an enjoyable and stimulating visit.

In June, we hosted the fifth annual symposium for the Cambridge Cancer Centre, with talks and posters from Cancer Centre members across a wide range of disciplines from physics to haematology, and an excellent plenary talk to round off the day by Fred de Sauvage from Genentech Inc. The Lecture Theatre was overflowing, with around 300 participants from across Cambridge (see page 84).

In October, we held the annual retreat, with two days of short talks, including case presentations to show how CI research has application in the clinic, and ‘breaking news’ presented by postdocs. I would like to thank the SAB, who have attended every retreat since the Institute was opened. They have provided valuable advice and support to me and to the junior group leaders, with whom they have met individually every year to discuss their progress.

The CI Symposium, continuing our now established theme of ‘Unanswered Questions in …’, focussed this year on cancer sequencing. Again, we had an outstanding set of speakers, lively discussion, and a record attendance of 240, including many from outside the UK (see page 82).

Finally, on December 10th, the CI was the setting for an announcement by the Prime Minister of the intention to allocate £100 million to sequence the genomes of patients with cancer and rare diseases. The PM was accompanied by David Willetts, MP (Minister of State for Universities and Science), George Freeman, MP (Life Sciences Adviser to the Government), and Professor Dame Sally Davies (Chief Medical Officer). The group were met and shown round the CI by Harpal Kumar (CEO, CRUK) and myself. The PM initiated a genome sequencing run in the genomics core facility, and everyone appeared thoroughly engaged with what they saw and heard.

This year was a busy one for the Cancer Centre. The Centre, in association with the CI, was accredited as a European Comprehensive Cancer Centre by the Organisation of European Cancer Institutes. The process, which required the collection of comprehensive information across the spectrum from research to the clinic, culminated in a two-day visit by the international panel. We have built on this process, and the discussion with the review panel, in drafting our strategy for the Cancer Centre which will form part of the 2013 Cancer Centre renewal. We continued our active programmes of public engagement. An Open Day was attended by 240 guests on 23 May. We have participated as always in fundraising talks with local groups, with visits by several of our scientists to speak to local sixth forms, and by participation in the Cambridge Science Festival (see page 85).

Notable individual successes include promotion to senior group leader for Masashi Narita, Doug Winton and Fanni Gergely. Shankar Balasubramanian and Tony Kouzarides (Gurdon Institute, CI adjunct faculty) were elected FRS; Kevin Brindle was elected FMedSci, and Shankar Balasubramanian and Eric Miska (Gurdon Institute, CI adjunct faculty) to EMBO. Shankar rounded off a remarkable year by also winning a Wellcome Trust Senior Investigator Award. Jason Carroll was jointly awarded the Future Leaders in Cancer Research Prize by CRUK – the prize is given to individuals who have completed their PhD within the last ten years and have demonstrated the potential to achieve world-leading status by producing international quality research.

Tiago Rodrigues, postdoc in the Brindle group, was chosen to be the Portuguese Olympic Attaché for the Olympic Games. PhD students recorded several achievements, which are listed in the PhD pages of the Report (page 86). They included the Kevin Burnand Prize of the Society for Academic and Research Surgery and the Poster of Distinction prize at the American Gastroenterological...
Association (Simon Buczacki, Winton lab); a British Society of Gastroenterology CORE/Dr Falk award (Richard Wells, Fearon lab); and membership of the Cambridge team that won the Biotechnology Young Entrepreneurs Scheme competition run by the BBSRC (Ajoob Baridi, Stingl lab). And not least, it is not only CI scientists that win awards. Our Property Services team, led by Martin Frohock, won the Association of Building Engineers Award 2012 in recognition of their use of best practice in building maintenance.

Finally, what are the consolidation of our science and the important changes that I mentioned in my opening sentence? The first change is that on 1st January 2013 the CI formally became part of the University Clinical School, although its core funding from CRUK will be on the same basis as before. This brings the CI into alignment with the other CRUK Institutes. A significant benefit will be that core funded research will now become eligible to attract government QR support, and the anticipated extra funds will contribute greatly to the future development of the CI, as well as to the Cancer Centre. Many of the Institute staff, ably led by our Director of Operations, John Wells, have worked hard all year to make the transfer possible.

The second change is that on 1st February 2013, I will step down as Director, to be succeeded by Simon Tavaré. Simon is already a Senior Group Leader in the CI, distinguished not only for his contributions to statistical and computational biology and most recently to genomic analysis, but also by his wide grasp of biological and clinical issues related to cancer research (see page 48). I personally very much welcome his appointment, which fits well with the emergence, as CI has developed over the past few years, of strong themes in gene regulation, clinical and functional genomics and imaging, each built on strong statistical and computational foundations. These will of course not be the only themes within CI going forward – there will also be cell biology and experimental cancer medicine to name but two. But I believe that they, and the appointment of the new Director, give the Institute a distinctive identity from which to build on our achievements in the first six years.

It has been a privilege to be the first Director and to have helped the Institute grow from its beginnings over ten years ago. Most of all it has been a privilege to work with such outstanding and supportive colleagues. Bruce Ponder

Guanine-rich DNA sequences that can adopt non-Watson-Crick structures in vitro are prevalent in the human genome. Whether such structures normally exist in mammalian cells has, however, been the subject of active research for decades. Here we show that the G-quadruplex-interacting drug pyridostatin promotes growth arrest in human cancer cells by inducing replication- and transcription-dependent DNA damage. A chromatin immunoprecipitation sequencing analysis of the DNA damage marker γH2AX provided the genome-wide distribution of pyridostatin-induced sites of damage and revealed that pyridostatin targets gene bodies containing clusters of sequences with a propensity for G-quadruplex formation. As a result, pyridostatin modulated the expression of these genes, including the proto-oncogene SRC. We observed that pyridostatin reduced SRC protein abundance and SRC-dependent cellular motility in human breast cancer cells, validating SRC as a target of this drug. Our unbiased approach to define genomic sites of action for a drug establishes a framework for discovering functional DNA-drug interactions.


Oestrogen receptor-alpha (ER) is the defining and driving transcription factor in the majority of breast cancers and its target genes dictate cell growth and endocrine response, yet genomic understanding of ER function has been restricted to model systems. Here we map genome-wide ER-binding events, by chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), in primary breast cancers from patients with different clinical outcomes and in distant ER-positive metastases (Figure 1). We find that drug-resistant cancers still recruit ER to the chromatin, but that ER binding is a dynamic process, with the acquisition of unique ER-binding regions in tumours from patients that are likely to relapse. The acquired ER regulatory regions associated with poor clinical outcome observed in primary tumours reveal gene signatures that predict clinical outcome in ER-positive disease exclusively. We find that the differential ER-binding programme observed in tumours from patients with poor outcome is not due to the selection of a rare subpopulation of cells, but is due to the FOXA1-mediated reprogramming of ER binding on a rapid timescale. The parallel redistribution of ER and FOXA1 binding events...
in drug-resistant cellular contexts is supported by histological co-expression of ER and FOXA1 in metastatic samples (Figure 2). By establishing transcription-factor mapping in primary tumour material, we show that there is plasticity in ER-binding capacity, with distinct combinations of cis-regulatory elements linked with the different clinical outcomes.


Solid tumors are heterogeneous tissues composed of a mixture of cancer and normal cells, which complicates the interpretation of their molecular profiles. Furthermore, tissue architecture is generally not reflected in molecular assays, rendering this rich information underused. To address these challenges, we developed a computational approach based on standard hematoxylin and eosin-stained tissue sections and demonstrated its power in a discovery and validation cohort of 323 and 241 breast tumors, respectively. To deconvolute cellular heterogeneity and detect subtle genomic aberrations, we introduced an algorithm based on tumor cellularity to increase the comparability of copy number profiles between samples. We next devised a predictor for survival in estrogen receptor-negative breast cancer that integrated both image-based and gene expression analyses and significantly outperformed classifiers that use single data types, such as microarray expression signatures. Image processing also allowed us to describe and validate an independent prognostic factor based on quantitative analysis of spatial patterns between stromal cells, which are not detectable by molecular assays. Our quantitative, image-based method could benefit any large-scale cancer study by refining and complementing molecular assays of tumor samples.


The expansion of repressive epigenetic marks has been implicated in heterochromatin formation during embryonic development, but the general applicability of this mechanism is unclear. Here we show that nuclear rearrangement of repressive histone marks H3K9me3 and H3K27me3 into nonoverlapping structural layers characterizes senescence-associated heterochromatic foci (SAHF) formation in human fibroblasts (Figure 3). However, the global landscape of these repressive marks remains unchanged upon SAHF formation, suggesting that in somatic cells, heterochromatin can be formed through the spatial repositioning of pre-existing repressively marked histones. This model is reinforced by the correlation of pre-senescent replication timing with both the subsequent layered structure of SAHFs and the global landscape of the repressive marks, allowing us to integrate microscopic and genomic information. Furthermore, modulation of SAHF structure does not affect the occupancy of these repressive marks, nor vice versa. These experiments reveal that high-order heterochromatin formation and epigenetic remodeling of the genome can be discrete events.


Prostate cancer (PC) is largely driven by androgens which signal through the androgen receptor (AR), leading to critical processes including glucose metabolism and cell proliferation. Despite currently-available treatments, including anti-androgens, many men develop castrate-resistant (CR) PC.

We have performed AR ChIP-seq in fresh tissue samples from men undergoing prostate surgery to study the transcriptional activity of the AR in a variety of patients with different stages of disease including early, localised disease and in particular those with advanced, CRPC. In contrast to previous published studies using cultured cells, this study of fresh human tissue has shown for the first time how other prognostic transcription factors (including STAT, E2F and MYC) might cross-talk with the AR in clinical samples, based on recruitment to DNA. We have found that the AR is repositioned in CRPC, including thousands of tissue-specific binding sites. Functional studies supported a model of altered signalling in vivo directing AR binding, with specific implications for our understanding and management of CR PC. We identified a 16-gene signature which outperformed a larger in vitro-derived signature in clinical datasets, showing the importance of persistent AR signalling in CRPC and revealing potential targets which would otherwise not
have been implicated in CRPC. As therapies are currently being developed to target c-MYC and STAT, this study may prompt additional combination trials whilst offering possible surrogate response markers.

Furthermore, this demonstration of the critical role of cellular context in the regulation of transcription factor target selection and gene regulation highlights a wider need to utilise clinical material for the study of oncogenic transcription factors.


Barrett’s esophagus is an example of a pre-invasive state, for which current endoscopic surveillance methods to detect dysplasia are time consuming and inadequate. The prognosis of cancer arising in Barrett’s esophagus is improved by early detection at the stage of mucosal carcinoma or high-grade dysplasia. Molecular imaging methods could revolutionize the detection of dysplasia, provided they permit a wide field of view and highlight abnormalities in real time. We show here that cell-surface glycans are altered in the progression from Barrett’s esophagus to adenocarcinoma and lead to specific changes in lectin binding patterns. We chose wheat germ agglutinin as a candidate lectin with clinical potential. The binding of wheat germ agglutinin to human tissue was determined to be specific, and we validated this specific binding by successful endoscopic visualization of high-grade dysplastic lesions, which were not detectable by conventional endoscopy, with a high signal-to-background ratio of over 5.


The elucidation of breast cancer subgroups and their molecular drivers requires integrated
views of the genome and transcriptome from representative numbers of patients. We present an integrated analysis of copy number and gene expression in a discovery and validation set of 997 and 995 primary breast tumours, respectively, with long-term clinical follow-up. Inherited variants (copy number variants and single nucleotide polymorphisms) and acquired somatic copy number aberrations (CNAs) were associated with expression in approximately 40% of genes, with the landscape dominated by cis- and trans-acting CNAs. By delineating expression outlier genes driven in cis by CNAs, we identified putative cancer genes, including deletions in PPP2R2A, MTAP and MAP2K4. Unsupervised analysis of paired DNA-RNA profiles revealed novel subgroups with distinct clinical outcomes, which reproduced in the validation cohort. These include a high-risk, oestrogen-receptor-positive 11q13/14 cis-acting subgroup and a favourable prognosis subgroup devoid of CNAs. Trans-acting aberration hotspots were found to modulate subgroup-specific gene networks, including a TCR deletion-mediated adaptive immune response in the ‘CNA-devoid’ subgroup and a basal-specific chromosome 5 deletion-associated mitotic network. Our results provide a novel molecular stratification of the breast cancer population, derived from the impact of somatic CNAs on the transcriptome.


Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden. Individual mutations have been probed using allele-specific assays, but sequencing of entire genes to detect cancer mutations in circulating DNA has not been demonstrated. We developed a method for tagged-amplicon deep sequencing (TAm-Seq) and screened 5995 genomic bases for low-frequency mutations. Using this method, we identified cancer mutations present in circulating DNA at allele frequencies as low as 2%, with sensitivity and specificity of >97%. We identified mutations throughout the tumor suppressor gene TP53 in circulating DNA from 46 plasma samples of advanced ovarian cancer patients. We demonstrated use of TAm-Seq to noninvasively identify the origin of metastatic relapse in a patient with multiple primary tumors. In another case, we identified in plasma an EGFR mutation not found in an initial ovarian biopsy. We further used TAm-Seq to monitor tumor dynamics, and tracked 10 concomitant mutations in plasma of a metastatic breast cancer patient over 16 months. This low-cost, high-throughput method could facilitate analysis of circulating DNA as a noninvasive “liquid biopsy” for personalized cancer genomics.

Kutter C, Watt S, Stefflova K, Wilson MD, Goncalves A, Ponting CP, et al. Rapid turnover of long noncoding RNAs and the evolution of gene expression. PLoS Genet. 2012; 8: e1002841. A large proportion of functional sequence within mammalian genomes falls outside protein-coding exons and can be transcribed into long RNAs. However, the roles in mammalian biology of long noncoding RNA (IncRNA) are not well understood. Few IncRNAs have experimentally determined roles, with some of these being lineage-specific. Determining the extent by which transcription of IncRNA loci is retained or lost across multiple evolutionary lineages is essential if we are to understand their contribution to mammalian biology and to lineage-specific traits. Here, we experimentally investigated the conservation of IncRNA expression among closely related rodent species, allowing the evolution of DNA sequence to be uncoupled from evolution of transcript expression. We generated total RNA (RNAseq) and H3K4me3-bound (ChIPseq) DNA data, and combined both to construct catalogues of transcripts expressed in the adult liver of *Mus musculus domesticus* (C57BL/6J), *Mus musculus castaneus*, and *Rattus norvegicus*. We estimated the rate of transcriptional turnover of IncRNAs and investigated the effects of their lineage-specific birth or death. IncRNA transcription showed considerably greater gain and loss during rodent evolution, compared with protein-coding genes. Nucleotide substitution rates were found to mirror the *in vivo* transcriptional conservation of intergenic IncRNAs between rodents: only the sequences of noncoding loci with conserved transcription were constrained. Finally, we found that lineage-specific intergenic IncRNAs appear to be associated with modestly elevated expression of genomically neighbours protein-coding genes. Our findings show that nearly half of intergenic IncRNA loci have been gained or lost since the last common ancestor of mouse and rat, and they predict that such rapid transcriptional turnover contributes to the evolution of tissue- and lineage-specific gene expression.
THE CI’S LABORATORIES UNDERTAKE RESEARCH IN FOUR MAIN AREAS:

1. Basic research into the cellular and molecular biology of cancer.
2. Research in molecular imaging, genomics, bioinformatics and biomolecular modelling.
3. Research focussed on specific cancer sites, which form a bridge between laboratory and clinic.
4. Clinical investigations including experimental medicine based clinical studies, conducted jointly with the University of Cambridge and National Health Service (NHS) clinical departments.
Recent advances in the understanding of nucleic acid function have shown that non-coding sequences and the chemical modification of nucleotide bases have key roles in regulating many cellular processes, from transcription and translation to cell division and genome stability.

Genetic information can be carried not only by the sequence of nucleic acids, but also by their structure and chemical modification. For example, guanine-rich sequences can form stable four-stranded structures called G-quadruplexes (G4), while certain cytosine bases in DNA can be methylated. We hypothesise that the formation of alternative nucleic acid structures, or the chemical modification of DNA, have critical functions in cancer and in normal cells. We aim to develop novel approaches that could be used in the treatment and diagnosis of cancer by identifying the location of base modifications in the genome of cancer cells and through the application of synthetic small molecules that selectively target alternative nucleic acid structures.

G-quadruplexes (G4)

In guanine (G)-rich regions, G bases can adopt stable intra-molecular arrangements to form four-stranded G4 structures comprising several stacked G-tetrads (Figure 1). Sequences with potential to fold into a G4 are common in the human genome and many are located within cancer-related genes such as KIT, RAS and SRC. G4 structures have been implicated in many biological processes from the control of cell division to the regulation of gene transcription, therefore we are seeking to understand their biological function and their validity as drug targets.

Figure 1


We have begun to identify where quadruplexes form within the genome and how this might be regulated in cancer phenotypes. We have synthesised small chemical probes and developed engineered antibodies that recognise G4 structures with high specificity and affinity. With these probes, we have visualised the formation of G4 structures in the nuclei of cancer cells (Figure 2). Using chromatin immunoprecipitation and next-generation sequencing (ChIP-seq) technologies we have located G4 structures in genomic DNA and have evidence that expression of G4-containing genes can be targeted by small molecule ligands. Our studies on cancer cells have also shown that our G4-binding small molecule, pyridostatin, induces the growth arrest of human cancer cells through the induction of a replication-and transcription-dependent DNA damage response. The sites of DNA damage were found to be in gene bodies in several oncogenes, including SRC (Figure 2). Pyridostatin treatment resulted in down-regulation of SRC expression and inhibition of SRC-dependent cellular motility. This work provides a novel framework for defining functional drug-DNA interactions for cancer therapies.

During cell division, chromosome ends (telomeres) are protected from damage by recruitment of a protective protein complex known as shelterin. Telomeres are capable of forming stable G4 structures in vitro and we have used our antibody probe to demonstrate their presence at telomeres and across the genome in chromosomes prepared from human cancer cells (Figure 3). Given that 85% of primary tumours show increased expression of telomerase, an enzyme required for telomere maintenance, targeting telomeres could lead to increased cancer cell death. Indeed, we have found that G4-binding small molecules can cause shelterin release from telomeres causing DNA damage to promote cell death. Telomeres are also actively transcribed into the G4-containing telomeric RNA (TERRA). While TERRA can form stable G4s in vitro, it is not known whether this is true in vivo or if it is needed for normal telomere biology. We have recently discovered that components in the shelterin complex bind the TERRA G4 structure, and we now aim to understand how this structure influences telomere function.

G4 structures are predicted computationally to be abundant in RNA, and their position suggests that they are associated with key aspects of RNA biology. We have reported that a conserved
RNA G4 motif in the 5’-UTR of the human NRAS oncogene can modulate protein translation, and can be targeted by small molecule ligands. However, it is not known how widespread G4 structures are in RNA transcripts, and what their functional relevance is. To address these questions, we have developed a novel procedure to synthesise small molecules with selectivity for RNA G4s. We are now using these and other chemical biology tools together with genome-wide technologies approaches to identify and map the existence of RNA G4 structures within the transcriptome and to investigate their role in cancer cells.

Epigenetics and Modified Bases
Chemical modifications to the bases in DNA can affect the activity of genes. These epigenetic marks switch genes on and off in different cell types, tissues and organs. The modified base 5-methylcytosine (5mC) is well known as an epigenetic mark that regulates transcription. Recently, three further modified bases, 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (Figure 4), have been discovered in the mammalian genome and are thought to arise from 5mC through the action of hydroxylases such as the TET-enzymes. Evidence is emerging that cancer cells have altered epigenetic profiles, yet the function of these modifications in normal cellular biology and their role in disease is not yet understood.

We are developing tools to locate and study the function of these modified bases using genomic technologies. We have recently created novel methods to quantitatively sequence both 5mC and 5hmC at single-base resolution and we are now focusing on the identification, mapping and elucidation of their biological function in normal and disease cells.

FOXM1
As cancer cells are often characterized by abnormal gene expression profiles, transcription factors represent an attractive target for intervention. In a separate project, we have been exploring whether transcription factors can be targeted by small molecules. Historically successful drug design for transcription factor targets has been limited as their surfaces are hydrophobic and flat, providing for few druggable regions. However, as direct DNA-protein contacts are made when their DNA binding domains (DBD) associate with DNA, small molecules that interact with the DBD to disrupt this complex could be promising. We have demonstrated the potential of this approach by examining FOXM1, a master regulator of cell cycle progression implicated in oncogenesis. We have found that the natural product thiostrepton binds to the FOXM1 DBD, resulting in dissociation of the DNA-transcription factor complex, and leads to genome-wide loss of FOXM1-binding in breast cancer cells. This work demonstrates the potential druggability of an important regulatory mechanism and paves the way for the rational design of more potent and selective agents that target FOXM1 and other transcription factors.

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Our laboratory focuses on discovering improved treatments for epithelial ovarian cancer using laboratory and clinical studies. Ovarian cancer has a high healthcare burden because of low cure rates and frequent recurrent disease that causes significant symptoms for patients. This is despite the fact that ovarian cancer is initially sensitive to systemic treatments and most patients are free of disease after completing initial surgery and chemotherapy. The fundamental problem that we are addressing is to understand how ovarian cancer cells escape initial treatment and the molecular mechanisms by which they acquire resistance to further therapy. Using genomic and functional studies we are identifying new biomarkers and treatment targets for testing in clinical trials.

Genomic studies of chemotherapy response in vivo
To identify genetic alterations that are selected for during the acquisition of drug resistance we are carrying out prospective clinical studies that collect cancer samples before and during neoadjuvant treatment. Our initial studies have focused on the drugs carboplatin and paclitaxel as these are the most important therapies in ovarian cancer. By using expression analysis and bioinformatics methods that have been developed to model the acquisition of resistance, we have identified clinically relevant biomarkers that overlap with independently identified genes from RNA interference screens (Swanton et al., Cancer Cell 2007; 11: 498).

Our studies depend upon having homogeneous patient cohorts with similar clinical characteristics. However, response to treatment in tumour masses can be heterogeneous and mixed response frequently occurs at different anatomical sites. For example, primary ovarian masses may respond better than peritoneal metastases. This differential response may be a result of variable blood supply and hypoxia that limits delivery and efficacy of chemotherapy.

We have confirmed these observations using functional magnetic resonance imaging for perfusion (Sala et al., Eur Radiol 2010; 20: 491) and diffusion and are now using imaging data to target the collection of tissues from responding and non-responding areas. This will allow us to calibrate genomic profiles much more precisely and to better identify the molecular determinants of resistance. High throughput sequencing with Illumina technologies is being used to quantitate expression and genomic changes and to identify novel fusion transcripts and mutations (Figure 1).

Differential sensitivity to paclitaxel as compared to carboplatin may depend on cellular pathways involved in maintaining chromosomal stability (CIN). To ask whether this may be clinically relevant we have tested surrogate expression markers of CIN in samples from a prospective neoadjuvant study and have shown that high measures of CIN predict resistance to paclitaxel and increased sensitivity to carboplatin (Swanton et al., PNAS 2009; 106: 8671) (Figure 2). Thus, measuring CIN pre-treatment may optimise choice of treatment for patients.

The key oncogenic and tumour suppressor genes for high-grade ovarian serous carcinoma have not been identified as this type of tumour has high rates of genomic instability, where many of the described alterations may be passenger mutations. Numerous studies have tested the association between TP53 mutations in ovarian cancer and prognosis but these have been consistently confounded by limitations in study design, methodology and/or heterogeneity in the sample cohort. To identify the true prevalence of TP53 mutations in high-grade pelvic serous carcinoma, we sequenced exons 2–11 and intron-exon boundaries in tumour DNA from 145 patients with invasive serous carcinoma of the ovary, fallopian tube and primary peritoneal cancer. Surprisingly,
pathogenic TP53 mutations were identified in 97% (n = 119/123) of HGS cases (Ahmed et al., J Pathol 2010; 221: 49). This is the first comprehensive mapping of TP53 mutation rate in a homogeneous group of high-grade pelvic carcinoma patients and shows that mutant TP53 is a driver mutation in the pathogenesis of HGS cancers.

Mechanisms of taxane resistance and the role of extracellular matrix
Taxanes, such as paclitaxel, interfere with the dynamic growth of microtubules by directly binding to them, leading to mitotic arrest and apoptosis. Paclitaxel is widely used to treat ovarian and breast cancers but drug resistance limits its clinical usefulness to only half of patients who receive it.

Alterations in the ratio of tubulin isoforms or mutations in tubulin can alter microtubule stability and sensitivity to taxane drugs. By studying cell line models of taxane resistance along with clinical samples we have recently shown that loss of the ECM protein, transforming growth factor beta induced (TGFBI), was sufficient to induce paclitaxel resistance in cells and ovarian cancer tissues (Ahmed et al., Cancer Cell 2007; 12: 514). We have also shown that TGFBI induces microtubule stabilisation that is dependent upon integrin-mediated FAK and RHO signalling pathways. Extracellular matrix proteins have been implicated in the acquisition of drug resistance in ovarian cancer although the mechanism by which this is achieved is unclear. Loss of TGFBI induces resistance by altering microtubules which are the direct pharmacodynamic target of paclitaxel. This work shows that the effects of ECM proteins on drug resistance may be very specific to particular cytotoxic treatments. As 30% of ovarian cancers do not express TGFBI, it may be an important biomarker for paclitaxel response.

Current projects are characterising how TGFBI interacts with integrins and other cell surface receptors and how this may be modulated therapeutically. It is now clear that TGFBI exerts its effects specifically through beta-3 integrins but is also co-regulated, and interacts with, other ECM proteins implicated in drug resistance. To identify the downstream pathways from FAK and RHO that alter microtubule stability, we have generated knock-out somatic cell lines using homologous recombination. These knock-out models have provided a powerful system to identify microtubule associated proteins responsible for effects on paclitaxel resistance. As TGFBI has complex roles in organising interactions between cells and ECM, we have studied its function in early development in Xenopus to identify how it may affect cell migration. Both loss and gain of function experiments have shown that TGFBI is required for somite development in Xenopus.

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The primary aim of our laboratory is to develop imaging methods that can be used in the clinic to detect early tumour responses to treatment. These could be used in early stage clinical trials of new drugs to get an indication of efficacy and subsequently, in the clinic, to guide therapy in individual patients.

Patients with similar tumour types can show markedly different responses to the same therapy. The development of new treatments would benefit, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment for a specific patient (Brindle, Nat Rev Cancer 2008; 8: 1).

**Imaging metabolism with hyperpolarised 13C-labelled cell substrates**

MRI gives excellent images of soft tissues, such as tumours. The technique works by imaging the distribution and MR properties of tissue water protons, which are very abundant (60–70 M in tissues). However, we can also use MR to detect metabolites in vivo. The problem is that these molecules are present at ~10,000 times lower concentration than the protons in tissue water, which makes them hard to detect and almost impossible to image, except at very low resolution. We have been collaborating with GE Healthcare in the development of a technique, termed “hyperpolarisation”, that increases sensitivity in the MRI experiment by more than 10,000x. With this technique we inject a hyperpolarised 13C-labelled molecule and now have sufficient sensitivity to image its distribution in the body and the distribution of the metabolites produced from it (reviewed in Brindle, Br J Radiol 2012; 85: 697).

Previously we had shown we can detect early response to chemotherapeutic drugs by monitoring decreased tumour utilization of one cell metabolite, pyruvate, and then detect subsequent cell death by watching the increased metabolism of another metabolite, fumarate. We are taking these forward in a clinical trial, which is due to start in 2013, to detect treatment response in lymphoma, glioma and breast cancer patients.

Treatment response with pyruvate is manifest as decreased lactate dehydrogenase, coenzyme and lactate concentrations. Subsequent cell death results in an increased conversion of hyperpolarised 13C-labelled fumarate into malate, due to increased permeability of the plasma membrane of the necrotic cell to fumarate. In the past year we have shown that these substrates can also be used to sensitively detect whether a tumour responds to an anti-angiogenic agent, Avastin, detecting decreased perfusion and cell death in a responding tumour and the effects of vascular normalisation in a largely non-responding tumour (Bohndiek et al, Cancer Res 2012; 72: 854). Fumarate was shown to be more sensitive for detecting diffuse cellular necrosis than diffusion-weighted MRI, which is used currently in the clinic to detect cell death. We have also shown that fumarate can be used to detect necrosis in other tissues, for example in the kidney, where we showed that hyperpolarised 13C-labelled fumarate could be used to distinguish acute tubular necrosis from inflammation (Clatworthy et al, Proc Natl Acad Sci USA 2012; 109: 13374).

A limitation of detecting treatment response with hyperpolarised [1-13C]pyruvate is that it must be administered in vivo at supra-physiological concentrations. This can be problematic, for example it can produce a transient decrease in tumour oxygenation. This problem can be avoided by using hyperpolarised [1-13C]lactate, which can be used at physiological concentrations. However sensitivity is limited in this case by the relatively small pyruvate pool size, which would result in only low levels of labelled pyruvate being observed even if there was complete 13C label equilibration between the lactate and pyruvate pools. We have developed a more sensitive method in which a doubly labelled lactate species can be used to measure lactate – pyruvate exchange in vivo. In this experiment exchange of the C2 deuterium label between injected hyperpolarized L-[1-13C,2-2H]lactate and endogenous unlabelled lactate is observed indirectly by monitoring phase modulation of the spin-coupled hyperpolarised 13C signal in a heteronuclear 1H/13C spin echo experiment (Kennedy et al, J Am Chem Soc...
The 1H reference image is a conventional image of tissue water protons and shows the tumour (at the bottom of the image). 13C chemical shift images acquired following i.v. injection of hyperpolarised [1-13C]dehydroascorbic acid (DHA) showed DHA throughout that region of the abdomen that was within the sensitive region of the surface coil, whereas the reduction of DHA to ascorbic acid (AA) occurred mainly within the tumour.

2012; 134: 4969). This experiment has the added advantage that only a single resonance is observed, which facilitates fast imaging.

Tumour cells up-regulate anti-oxidant metabolic pathways in order to deal with the high levels of oxidative stress to which they are exposed. We reported last year that we could use hyperpolarised 13C-labelled vitamin C (ascorbic acid) and its oxidised product, dehydroascorbic acid, to image tumour redox status in vivo, and have subsequently demonstrated that tumour tissue is very efficient at taking up and re-reducing dehydroascorbic acid to ascorbic acid (Figure 1).

Tumours have long been known to contain high levels of neutral lipid in the form of cytoplasmic lipid droplets. However, the metabolic origin of these has not been clear. We showed this year that these accumulate due to stress-induced mitochondrial oxidative damage, which results in a decrease in mitochondrial fatty acid oxidation and a consequent increase in the synthesis of triacylglycerols, which accumulate in the lipid droplets (Boren and Brindle, Cell Death and Diff 2012; 19: 1561). Since these lipid droplets give rise to a relatively intense signal that we can detect using MRI techniques this has provided us with a new way of non-invasively detecting the very earliest signs of cell stress (within hours of drug treatment) in tumours, and possibly in other tissues as well.

**Future directions**

Aberrant glycosylation is a hallmark of cancer. We showed last year, in a collaboration with Rebecca Fitzgerald’s group in the Hutchison/MRC Research Centre, that we could use a fluorescently-labelled lectin for endoscopic detection of early dysplasia in the oesophagus (Bird-Lieberman et al., Nat Med 2012; 18: 315). We also developed a novel method for assessment of tumour glycosylation state, in which sugar analogues are incorporated metabolically by tumour cells in vivo and detected subsequently by a highly selective chemical reaction (“click chemistry”) with an imaging probe. We will conduct further studies with fluorescently labelled lectins in the oesophagus and the colon and will use our newly developed click reagents to obtain better glycan image contrast in vivo.

We will continue development of a targeted radionuclide and fluorophore-labelled imaging agent for detecting cell death, with a view to commercial development in the preclinical arena and also translation to the clinic. We will continue with the development and application of new hyperpolarised 13C labelled metabolic tracers and will take the first steps in translating hyperpolarised 13C technology to the clinic with the installation of a clinical polariser in the Department of Radiology.

**Publications listed on page 68**
We have recently characterized a new integrative classification of breast cancer into 10 subtypes, with significant implications for personalized cancer management and the study of the biology of the disease. Our laboratory continued its work on both of these fronts.

Translational breast cancer genomics: applications of molecular profiling in prognosis, prediction and novel therapeutics

The new genome-driven classification of breast cancer into 10 subtypes resulted from the analysis of the genomic and transcriptomic landscapes of 2000 breast cancers with linked clinical follow-up. The joint clustering of CNAs and gene expression profiles resolves the considerable heterogeneity of the expression-only subgroups (luminal, basal-like and Her2), and highlights in particular several novel ER+ groups and a genomically quiescent group (CNA-devoid) made up of both ER+ and ER- cases. We are now asking the question whether ER-binding at the whole genome level differs between the ER+ groups and whether the pattern of point mutations in the CNA-devoid group is distinct and contributing to the prominent immune response seen in a subset of these tumours. Having characterized miRNA expression profiles from ~1,300 of the same cases we have now integrated the three levels of data such that we could: define the contribution of genomic, transcriptional and post-transcriptional events to miRNA expression patterns; characterize the impact of clinical and histopathological variables on miRNA expression profiles; define key cellular pathways intertwined with the miRNA landscape (for example TGFβ and Wnt); demonstrate an apparent central role for miRNAs in the biology and outcome of CNA-devoid breast tumours; and unravel what appears to be the role of a miRNA network as a modulator of immune response in CNA-devoid tumours. We have sequenced TP53 in 1500 of the same cases and this has revealed differences of mutation frequency and mutation type between the ten integrative clusters, providing further evidence for their distinct biology. We are currently sequencing 200 candidate driver genes in all 2000 cases and this will provide us with a landscape of drivers for each subtype.

We have continued our collaborative efforts in both systems pathology and digital pathology, showing that image analyses algorithms adapted from astronomy can be robustly deployed for automated analysis of tissue protein expression.

We further expanded the tissue microarray (TMA) resource, which now includes ~11,500 samples from a population-based cohort and from five randomised clinical trials. We have continued to use this unique resource of clinically-annotated tumours to perform studies that rigorously conform to the REMARK (REporting recommendations for tumour MARker prognostic studies) guidelines. For example, we characterized the biological and prognostic associations of miR-205 and let-7b using ISH, and we have shown that Aurora kinase A outperforms Ki67 as a prognostic marker in ER+ breast cancer.

Collaborators: Sam Aparicio (University of British Columbia), Simon Tavaré, Jason Carroll and Florian Markowitz (CRUK CI), Paul Pharoah (Strangeways Research Laboratory), Mike Irwin and Nick Walton (Institute of Astronomy), Anne-Lise Børresen-Dale (Oslo)

Functional breast cancer genomics: characterising tumour initiating/cancer stem cells in breast cancer subtypes

We have significantly improved our protocols for mammosphere assays, limiting dilution analysis of tumour initiating cells and xenografting of primary tumours (Figure 1). This gives us the ability to work with primary cells that can be tested both in vitro and in vivo using therapeutic and functional perturbations. We will continue to use these resources to study the roles of TGFβ, miRNAs and small molecules in the different breast cancer subtypes.

Collaborator: John Stingl (CRUK CI)

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Figure 1

*In vitro* growth of patient derived tumour cells. Representative images of primary cells from engrafted patient-derived breast cancer tissue. Xenograft tumour fragments were dissociated and single cells plated in ultra-low attachment plates in specific growth media conditions.
We are interested in defining the genomic and molecular features of oestrogen receptor (ER)-mediated transcription in breast cancer cells. We are specifically interested in understanding how these events and the machinery involved cause breast cancer cells to grow.

Oestrogen receptor is the defining feature of luminal breast cancers, where it functions as a transcription factor to induce cell cycle progression. ER is also the target of most endocrine therapies, including tamoxifen and aromatase inhibitors, which are effective treatments. However, some women can develop resistance to these drugs and in many cases, ER simply gets switched back on again, despite the presence of the drug. ER transcriptional activity requires a number of co-factors and co-operating transcription factors that possess enzymatic activity to alter chromatin structure, the outcome of which determines transcriptional activity. It is currently known that a number of ER co-factors can either assist in transcription (including SRC-1 and AIB-1) or are involved in gene repression by tamoxifen (including N-CoR and SMRT).

In addition, it is now known that ER requires proteins called pioneer factors to be able to maintain its association with DNA. Two of these proteins are FoxA1 and GATA3. FoxA1 is required for all ER-DNA interactions and in the absence of FoxA1, ER does not associate with DNA, switch genes on or cause cells to grow. Importantly, FoxA1 is also required for growth of cells that have acquired resistance to standard therapies, such as tamoxifen. Therefore, FoxA1 constitutes an attractive drug target for women with drug resistant breast cancer. Recent discoveries show that both GATA3 and FoxA1 are mutated in a significant fraction of women with breast cancer, but we do not currently know what the functional consequences of these changes are.

Characterisation of the role of GATA3 in ER biology

We are interested in identifying and characterising the role of the putative pioneer factor GATA3 in regulating ER activity. Using transcription factor mapping techniques (ChIP-seq), we have identified all the GATA3 binding events in a breast cancer cell line. This reveals high overlap with ER, supporting the hypothesis that GATA3 is intimately involved in ER function. For the first time, we have specifically removed GATA3 and assessed the impact on ER function in breast cancer cells. Unlike FoxA1, which is required for all ER-DNA interactions, we find that loss of GATA3 results in inhibition of some ER binding events, and also results in the reprogramming of novel ER binding events not normally seen in the presence of GATA3. These new ER-DNA interaction regions only observed in the absence of GATA3 correlate with changes in the genes that are regulated by ER, showing that the novel ER binding events are functionally relevant for breast cancer cells. Since GATA3 is mutated in more than 10% of all breast cancers, we believe that alterations in GATA3 sequence fidelity may be impacting ER binding capacity and the target genes that are regulated by ER. We are currently exploring what specific impact GATA3 mutations have on breast cancer cell growth and drug response.

Genomic analysis of ER function in primary breast cancer

All ER genomic studies to date have been limited to breast cancer cell line models, yet they have revealed extraordinary features about ER biology. We have now been able to extend genomic transcription factor mapping experiments into frozen primary breast cancer samples, by performing ER ChIP-sequencing in luminal breast cancer material. The data confirm that ER ChIP-seq can be performed in primary breast cancer samples and that the ER binding events accurately represent the binding sites in the cell lines. However, there are significant numbers of ER binding events that are acquired in tumours with a poor clinical outcome and in metastatic material that originated from an ER positive breast cancer. The novel ER binding events correlate with genes that have predictive value in independent breast cancer cohorts. We can model these events using drug sensitive or resistant cell line models, where ER binding events are dynamic and can be reprogrammed with growth factor stimulation. The reprogrammed ER binding events are mediated by changes in the pioneer factor FoxA1. We are currently exploring what enables changes in FoxA1, since these mediate the changes in ER binding events and subsequently...
influence the transcriptome. In addition, in close collaboration with the Caldas laboratory, we are embarking on a large scale transcription factor mapping experiment to identify ER and FoxA1 binding events by ChIP-seq, in ~200 primary breast cancers with detailed transcriptomic information and clinical follow up.

Understanding the role of androgen receptor in breast cancer
We recently showed that a subset of breast cancers, called molecular apocrine tumours, are driven by the male hormone receptor androgen receptor (AR) instead of ER. Unexpectedly, AR simply substitutes for ER and gets recruited to the same sites in the genome and subsequently regulates the same genes normally switched on by ER. We had also made the observation that FoxA1 was mediating AR-DNA interactions, which paralleled the events normally seen between FoxA1 and ER. More recently we have explored the requirement for FoxA1 in AR-DNA binding capacity in molecular apocrine breast cancer cells. We find that specific loss of FoxA1 results in a reprogramming of AR to new regions in the genome. This is similar to what is observed in prostate cancer models and also what we observed between GATA3 and ER in breast cancer. Therefore, in molecular apocrine breast cancer, AR can mimic ER to regulate the genes normally controlled by ER, but its ability to move around the genome in the absence of FoxA1 is closer to what is observed in the prostate context. We are currently exploring the underlying mechanisms that govern AR reprogramming in the absence of FoxA1 and the potential impact that FoxA1 mutations may have on this process in breast cancer.

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Figure 1
Changes in ER function and DNA structure when GATA3 is specifically removed from breast cancer cells. GATA3 was specifically inhibited (siGATA3) or a control was included (siControl) and global mapping was conducted on ER, the ER co-activator p300 and two histone marks (H3K4me1 and H3K27Ac). For each transcription factor or histone mark, the two conditions are shown. An example of a genomic region encompassing the estrogen induced gene TFF3 is shown. Loss of GATA3 results in increased recruitment of the co-activator p300 and changes in the distribution of H3K4me1 and H3K27Ac, which are indicators of gene expression. This correlates with increased expression of the TFF3 gene (not shown).
TUMOUR IMMUNOLOGY AND THE FAP+ STROMAL CELL
www.cruk.cam.ac.uk/douglasfearon

Even though spontaneous or vaccine-induced systemic immune responses to cancers occur, the stromal microenvironment of tumours protects cancer cells from immune attack. We have recently found that a stromal cell identified by its expression of fibroblast activation protein-α (FAP) mediates immune suppression in murine tumours. We seek ways to block its immune suppressive functions to improve clinical tumour immunotherapy.

The FAP+ stromal cell in tumours
The proposal of the immune surveillance of cancer, as put forward by Macfarlane Burnet and Lewis Thomas, hypothesizes that cancers may sufficiently differ from normal cells so that they would be recognized by the immune system and eliminated. Today we know that cancers, either because they are virally induced and express foreign viral antigens, or are genetically unstable and express mutated self antigens, do induce systemic immune responses, but we also recognize that cancers usually escape immune control.

Two general mechanisms have been proposed for the ability of cancers to circumvent an immune response: establishing an immune suppressive microenvironment within the tumour, and the generation and immune selection of cancer cell variants that are not immunologically recognized. Evidence for both exists, but we decided to concentrate on immune suppression because it would dominate over immune selection, and it offered the possibility of therapeutic approaches.

Early evidence for an immune suppressive microenvironment within tumours was the observation that an established tumour, containing not only cancer cells but also non-cancer “stromal cells”, was resistant to killing by tumour antigen-specific T cells. However, cancer cells alone, without an accompanying stroma, were eliminated. This finding was made more than a quarter of a century ago, but the realization that attention must be directed to the tumour stroma has been slow to develop, perhaps for three reasons: a continued emphasis on the possibility that the cancer cell itself was responsible for tumoural immune suppression, gaps in our understanding of how the immune system worked, and the complexity of the tumour stroma.

The tumour stroma is comprised of three general cell types, those involved with forming the tumour vasculature, cells of the innate and adaptive immune systems, and mesenchymal cells, or fibroblasts. Most work has been directed to understanding the roles of the cells of the immune systems, with the reasonable rationale that the processes intrinsic to this system, which control auto-immunity, also would be involved in immune suppression in the tumour microenvironment. This approach has been productive and has led to the development of a clinically approved treatment for metastatic melanoma, ipilimumab, an antibody that blocks the function of CTLA-4, a lymphocyte receptor. This treatment, however, causes systemic autoimmunity because it does not selectively target immune suppression in the tumour microenvironment.

To determine the cellular basis for immune suppression within the tumour microenvironment, we focused on stromal cells of mesenchymal origin, which have usually been referred to as myofibroblasts or carcinoma-associated fibroblasts (CAFs). These cells have been examined for their ability to promote tumour growth, but not by an immunological mechanism. Over the last 20 years, however, an interesting correlation was found between the occurrence of chronic inflammatory lesions of various types, such as atherosclerosis, rheumatoid arthritis, cirrhosis, and dermal scars, and the presence of a mesenchymal cell that was first observed in most human adenocarcinomas by its expression of a membrane protein, FAP.

The recognition that tumours contain the same inflammatory cells that characterize these chronic lesions, the likelihood that these lesions represent attempts at tissue repair, and the possibility that immune suppression is a normal component of tissue repair led to the consideration that the FAP+ stromal cell might have a role in tumoural immune suppression.
We tested this possibility by developing a mouse line in which the primate diphtheria toxin receptor is expressed in FAP+ stromal cells to enable their conditional depletion by the administration of diphtheria toxin. The experiment was informative in that depleting FAP+ cells from the stroma of established tumors caused immune control of tumour growth. This finding was initially made with immunogenic, ectopic tumours caused by injecting cultured cancer cells, but has been extended now to the Tuveson laboratory model of spontaneous pancreatic ductal adenocarcinoma in which cancer cells express mutant Trp53R172H and KrasG12D alleles (Figure 1). The FAP+ stromal cell is a non-redundant element of tumoural immune suppression, and the presence of these cells in human adenocarcinomas suggests that these findings in the mouse may be relevant to human cancer.

The FAP+ stromal cell and normal tissues
The possibility that FAP+ stromal cells might have functions in normal tissues was raised by their presence in the somites of developing mouse embryos, and in the uterus and placenta. To examine this possibility, we developed a mouse in which luciferase was expressed in FAP+ cells, which has revealed that FAP+ cells are present in almost all tissues of the adult mouse. Thus, FAP expression may denote a mesenchymal lineage with both shared and tissue-specific homeostatic functions, as well as its immune suppressive function in tumours, which may be an elicited activity that is potentially available to “injured” tissues throughout the body.

We have begun to define the functions of FAP+ cells in several normal tissues, including skeletal muscle in which we have shown that they are required for the maintenance of normal muscle mass. Remarkably, cancer may also affect some tissue homeostatic functions of FAP+ cells, in that in two mouse models of cancer-induced cachexia, which is the loss of skeletal muscle mass that may occur independently of food intake, FAP+ cell numbers are decreased in skeletal muscle, perhaps accounting for the cachexia. Cachexia is a serious clinical problem, and these findings may lead to an improved understanding of this process.

Next steps
The depletion of FAP+ cells is not a reasonable option for enhancing the ability of the immune system to control tumour growth because they are necessary for the functions of normal tissues. Therefore, we must determine the molecular basis of the immune suppressive function of the tumoural FAP+ cell, and develop therapies that will interrupt this function. Our strategy is to identify among the genes that are selectively expressed in the tumoural FAP+ cells candidates for immune suppression. We are also determining how FAP+ cells accumulate in the tumour. Conceivably, they may be generated by replication from FAP+ cells in the local tissue, or they may come from another site, such as the bone marrow, where we have shown them to proliferate. Either of these two research directions may lead to therapeutic opportunities for enhancing immune control of tumour growth.

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The work in our laboratory focuses on the centrosome, an organelle best known for its role as a major microtubule organising centre.

Emerging evidence, however, suggests that the centrosome also acts as a communication hub that spatially concentrates diverse signalling pathways. While centrosome number and function are strictly regulated within healthy cells, tumours display a multitude of centrosome abnormalities. How such anomalies contribute to tumourigenesis is an important and as yet unresolved question.

In most normal cells the centrosome is composed of a pair of cylindrical structures, the centrioles, which are embedded in an electron-dense amorphous matrix, the pericentriolar material. The latter provides the site for microtubule nucleation and therefore strongly influences microtubule numbers and organisation throughout the cell cycle. Proteomic studies of whole human centrosomes suggest that the organelle contains up to 300 proteins, many with unknown function. Like DNA, the centrosome duplicates in S-phase. This process is tightly controlled, since abnormal centrosome numbers cause mitotic spindle defects, culminating in mitotic catastrophe or loss of genome stability.

Tumours exhibit a wide variety of centrosome abnormalities that range from numerical and structural, to functional and positional aberrations. It is not well understood how these abnormalities arise in cancer and how they contribute to tumourigenesis. We have two basic goals in the laboratory. First, we want to address how centrosome biogenesis and function are controlled in normal cells. In addition, our studies will provide insight into how deregulation of particular centrosomal proteins affects cell growth, mitotic spindle formation and genome stability. Second, we want to identify the molecular mechanisms and signals that govern centrosome positioning and microtubule nucleation in response to environmental cues. These goals aim to provide insight into basic biological processes whose de-regulation is implicated in the development of cancer.

Centrosomes, genome stability, developmental defects and cancer
Centrosomes duplicate once and only once per cell cycle. In brief, the process involves the formation of a single procentriole next to each parental centriole and its subsequent elongation. While the morphological changes that occur during centrosome duplication are well documented, our understanding of the molecular pathway responsible for the timely assembly of one and only one procentriole per parental centriole in each cell cycle is still far from being complete. Our recent work revealed a new regulator of procentriole formation – the core centrosomal protein, CEP63. We generated cells that lack functional CEP63. CEP63 mutant cells grew more slowly than control cells and displayed a high incidence of monopolar spindles as a result of abortive centrosome duplication cycles. We subsequently discovered that CEP63 formed a molecular complex with CEP152, an evolutionarily highly conserved protein required for initiating procentriole assembly. Using super-resolution microscopy we were able to visualise the sub-centrosomal localisation of the CEP63-CEP152 complex.
complex. The two proteins form a discrete ring-shaped structure at the proximal end of parental centrioles, seemingly occupying a space near the centriole walls, a site implicated in both procentriole formation and centriole cohesion (Figure 1). Our study revealed that the role of CEP63 in centrosome duplication is to enrich and organise CEP152 at the centrosome. To provide further insight into the function of CEP152 in vertebrate cells, we disrupted the gene in cells. Characterisation of the CEP152-knockout cell line revealed that they lack intact centrosomes. This finding is particularly exciting, since this seems to be the first vertebrate cell line that survives in the absence of these organelles. We subsequently disrupted another gene that encodes a core centriole assembly protein, generating further cell lines without centrosomes. Utilising these cell lines as the main tools, our current work focuses on dissecting the precise function of centrosomes in proliferating vertebrate cells – from signalling to mitotic spindle assembly. Our results indicate that vertebrate cells grow more slowly and assemble abnormal mitotic spindles in the absence of intact centrosomes (Figure 2).

Remarkably, inherited mutations in CEP63 and CEP152 lead to loss of genome stability and severe developmental defects in humans. How these centrosomal proteins are involved in mammalian development is not well understood. It is therefore crucial to model the physiological effects of these mutations in organisms. Indeed, our recent work has uncovered a central role for centrosomes in genome stability and body growth in mice (MacIntyre et al, PLoS Genet 2012; 8: e1003022). We are concentrating our efforts on elucidating the specific mechanisms by which centrosome abnormalities cause growth defects in organisms. Our goal is to translate this knowledge into understanding how aberrant centrosomes could contribute to tumourigenesis.

Centrosome segregation
The centrosome duplication cycle is carefully coordinated with DNA replication, with many regulatory molecules shared between the two processes. However, to maintain normal centrosome numbers in cells, correct centrosome duplication must always be followed by equal segregation of the two centrosomes into the daughter cells. Centrosome segregation, just like chromosome segregation, is entirely dependent on a functional mitotic spindle. Centrosomes occupy a position at the spindle pole during mitosis, and the centrosomes are transmitted to the daughter cells by means of their attachment to spindle poles. Therefore a close association must be maintained between these structures throughout mitosis for normal centrosome segregation to occur. To understand how this is achieved, we need to elucidate the molecular complexes that build the centrosome-spindle pole interface. We have previously identified a role for the conserved protein CDK5RAP2 in contributing to this interface and hypothesised that CDK5RAP2 might act in a large protein complex. Indeed, to date several interacting proteins, including microtubule stabilising and cross-linking factors, have been discovered in our laboratory. Using genetic and biochemical models, we are currently investigating how the concerted efforts of these molecules ensure that centrosomes remain attached to spindle poles in the presence of complex spindle forces.

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Magnetic resonance imaging and spectroscopy (MRI and MRS) have many uses in cancer research. We use these methods, both in the laboratory and in patients, to study basic cancer biology, to improve non-invasive methods for tumour diagnosis and grading, to personalise patient therapy, and to develop biomarkers for monitoring the action of anticancer drugs.

Sidhartha Nagala, a PhD student supervised by Jonathan Gillard (Department of Radiology) has completed a study on biochemical characterization of human thyroid tumours using HRMAS 1H NMR.

Sara Dietz completed her PhD (jointly supervised by Colin Watts, Department of Neurosurgery) on human glioblastoma multiforme cancer stem-like cells. When they differentiate into cancer cells there are marked metabolic changes, most of which reverse when they de-differentiate. Sara also studied the metabolomic characteristics of glioblastoma cells stained with 5-aminolevulinic acid, a marker used for fluorescence-guided resection (Piccirillo et al. Br J Cancer 2012; 107: 462).

Preclinical MRI and MRS

Preclinical MRI and MRS studies are led by Dominick McIntyre, with Davina Honess. Leanne Bell completed her PhD on the dense collagenous tumour matrix of the drug-resistant KPC pancreatic tumour. To monitor drugs that break down that matrix and enhance drug sensitivity, Leanne monitored tumours by dynamic contrast enhanced MRI (DCE-MRI) and magnetisation transfer MRI (MT-MRI) in vivo, and then, ex vivo, by second harmonic generation (SHG) imaging of collagen (with Lorraine Berry, Microscopy Core). Transplanted KPC tumours have very little collagen, but when transplanted with pancreatic stellate cells these tumours form collagen deposits; MT-MRI non-invasively detects differences between spontaneous and allograft KPC tumours (Figure 1).

Nicola Ainsworth, a clinical research fellow (jointly supervised by Jonathan Gillard, Department of Radiology, and in collaboration with Susan Harden, Department of Oncology) is studying the metastasis of small cell lung cancer (SCLC) to the brain. Up to half of patients with SCLC will develop cerebral metastases, but since we cannot predict which ones, the current practice is to give them all prophylactic cranial
Different *in vivo* models of pancreatic ductal adenocarcinoma (PDA) have different collagen content, as probed by *in vivo* magnetisation transfer MRI (MT-MRI) and *ex vivo* second harmonic generation (SHG) microscopy.

(A-D) SHG microscopy data:

- (A) Spontaneous KPC tumours have large collagen deposits.
- (B) Transplanted PDA tumours have very little collagen.
- (C) When transplanted with pancreatic stellate cells, PDA tumours form many collagen deposits.
- (D) Quantitative SHG: fibrotic allograft KPC tumours have significantly less collagen than spontaneous KPCs but more than allografts. (E) *In vivo* MT-MRI is sensitive to differences in collagen content between spontaneous KPC tumours and transplanted PDA tumours (Bars represent mean ± SEM).

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irradiation, a therapy with significant long-term side effects. In addition to clinical studies (see below) she has developed a preclinical model of brain metastasis to investigate biological correlates of MRI signal changes.

The Griffiths (Dominick McIntyre and Davina Honess) and Brindle (Dmitry Soloviev and David Lewis) laboratories are jointly participating in the pre-clinical element of QuIC-ConCePT, a multi-centre EU-funded project under the Innovative Medicines Initiative. The aim is to qualify existing imaging methods for use in anticancer drug trials. Postdoc Kathrin Heinzmann is assessing diffusion-weighted MRI (DW-MRI) and ¹³C-fluorothymidine PET for their ability to identify drug-induced necrosis and changes in proliferation, respectively, in the KPC pancreatic tumour model.

**Clinical MRI and MRS**

Mary McLean leads our work on tumours in patients. We collaborated with James Brenton (CI) and Evis Sala (Radiology) in an MRI and MRS study on the response of ovarian cancer to chemotherapy (Sala et al, *Radiology* 2012; 263: 149). DWI-MRI was the best and quickest marker of tumour response. Another collaboration with Evis Sala, Vincent Gnanapragasam (Surgery) and David Neal (CI) used DWI, DCE-MRI and MRS to predict and detect response to androgen deprivation in advanced prostate cancer (Barrett et al, *Magn Reson Med* 2012; 67: 778). Current work includes investigating magnetization transfer contrast for characterizing heterogeneity in the microenvironment of pelvic tumours. We have also begun using MRI to investigate response to the new chemotherapy agent bevacizumab in breast cancer, as part of the ARTemis clinical trial in collaboration with Carlos Caldas (CI), Helena Earl (Oncology), and Fiona Gilbert (Radiology).

Tumours avidly convert glucose to lactic acid, so lactate concentration could be a biomarker of tumour activity; we therefore investigated its measurement reproducibility in brain tumours (McLean et al, *J Magn Reson Imaging* 2012; 36: 468), and we are collaborating with General Electric to further improve the discrimination of lactate from overlapping lipid signals. Nicola Ainsworth is recruiting SCLC patients into a study (CLUB-01), in which they are imaged before and after prophylactic cranial radiotherapy. An MPhil student, Anna Brown, has developed texture analysis software for detection of subtle changes in these brain images that may be apparent during the formation of early metastases.

These texture analysis techniques have also been applied by Siddhartha Nagala who has been testing MRS, MT-MRI and DWI for the diagnosis of cancer in follicular thyroid nodules and parotid lumps. Texture analysis of the DWI was able to discriminate the benign thyroid nodules from the malignant ones with excellent sensitivity and specificity, demonstrating that a larger study is warranted.

Publications listed on page 71
The aims of the Pharmacology and Drug Development Group (PDDG) are to optimise the pre-clinical development and science-led clinical application of novel therapies, including ‘first into man’ (phase I) studies.

We use pre-clinical model systems to inform the early clinical development of novel agents and identify interesting drug combination strategies. The PDDG is closely linked with the Early Phase Clinical Trials Team (EPCTT) based in the Cambridge Cancer Trials Centre, at Addenbrooke’s Hospital, allowing us to implement our laboratory findings in the clinic.

In the laboratory, we generally use model systems representing pancreatic cancer, which complements our clinical links: Duncan Jodrell is a member of the team at Addenbrooke’s who care for patients with pancreatic cancer, and is also the Director of the Cambridge Pancreatic Cancer Centre (www.cambridge-pcc.org). Pancreatic cancer is also a major unmet clinical need and a priority cancer with gemcitabine to patients with metastatic pancreatic adenocarcinoma.

One example of this approach is our studies using novel Aurora kinase inhibitors. The Aurora family of serine/threonine protein kinases play a critical role in cell division, with key roles in the mitotic spindle checkpoint. Aurora kinase-A (AK-A) has been identified as a cancer susceptibility gene, and elevated expression levels of AK-A are detected in many different types of cancer. In addition, AK-A over-expression is associated with resistance to taxanes. In cell culture models of pancreatic (Lin et al, Br J Cancer; 2012; 107: 1692) and urothelial cancers, we have demonstrated, using a novel Aurora A specific inhibitor, regions of both synergy and antagonism in growth inhibition combination surfaces and we are now undertaking in vivo experiments to test these findings further.

In general, it is assumed that combinations of agents have similar effects on normal and tumour cells, but this is not always the case. An optimal combination would lead to synergy in cancer cells and antagonism in normal cells, reducing the toxic side effects that often limit dosing. In studies with normal myeloid precursors (CFU-GM) and other diploid cells (e.g. IMR90 fibroblasts), we have demonstrated that the synergistic effects of combining an AK-A inhibitor and paclitaxel are not seen in non-malignant cells. This project is also utilising a mathematical model of the spindle assembly checkpoint to predict drug effects, through collaboration with Bob Jackson (Pharmacometrics Ltd). We ultimately intend to extend our pre-clinical findings into clinical trials.
Another example of the application of these combination approaches is the evaluation of a novel inhibitor of the cell cycle regulator CHK1, in combination with gemcitabine (a collaboration with Sentinel Oncology). Synergy has been observed in pancreatic cancer cell lines in vitro, and the effect is now being investigated in mouse models of pancreatic cancer.

As a result of our collaboration with Steve Ley and Rebecca Myers (Department of Chemistry) and Fanni Gergely (CI), a novel, selective allosteric inhibitor of the kinesin motor protein HSET has been synthesised and evaluated biologically (CW069). Using live cell imaging and confocal microscopy, we have identified phenotypic changes demonstrated previously to be related to HSET knockdown. This compound represents the first selective inhibitor of HSET and we hope, following further physicochemical analysis and synthetic efforts, that it may lead to identification of a candidate molecule for pre-clinical development.

Our collaboration with Gillian Murphy (CI) continues to investigate TNF-α converting enzyme (TACE, ADAM17) as a therapeutic target. In the evaluation of a novel antibody targeting ADAM17, favourable pharmacokinetics and encouraging pharmacodynamic read-outs have been achieved (Richards et al, PLoS ONE 2012; 7: e40597) and efficacy studies are ongoing.

In a new collaboration with Doug Fearon (CI), we are studying two diverse features of pancreatic adenocarcinoma (cancer-related cachexia and local immunosuppression in the tumour) which appear to be related to a particular cell lineage found in tumour cells and skeletal muscle. We hope that both these projects will lead to novel therapeutic interventions in this disease, which will feed into the growing portfolio of the EPCTT.

Currently, the EPCTT is supporting 12 experimental medicine studies that are actively recruiting patients; two combination phase I trials, a further six single agent phase I trials, two biomarker trials and two PK studies. We are continuing to explore novel PET and MR approaches in our trials and look forward to initiating a series of trials supporting the clinical development of hyperpolarised $^{13}$C pyruvate based PD studies (in collaboration with Kevin Brindle and Ferdia Gallagher). Nine further protocols are in the set-up phase for initiation in 2013.

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The Markowetz lab develops algorithms and statistics to leverage complex and heterogeneous data sources for biomedical research. Our main research question is: How do perturbations to cellular mechanisms shape phenotypes?

Understanding the mechanisms underlying risk genes
Different people have different genetic variants, and some of them are at a higher risk of developing cancer than others. Genome-wide association studies have identified many low-penetrance risk variants for complex diseases such as breast cancer. However, individually the identified variants have only small effects and cancer GWAS have been criticised for the lack of novel biological insights gained from the identification of these variants. To overcome these limitations we have worked with Bruce Ponder’s lab on a systems biology approach to study the architecture of the gene networks in breast cancer that are associated with FGFR2, the top GWAS ‘hit’.

Our study has given novel insights into the FGFR2 response and sheds light on the contribution of common variants to breast cancer risk: We find that risk SNPs, including those that do not reach genome-wide significance, map preferentially near FGFR2 responsive genes. This suggests that at least some of these SNPs are functional and contribute to the “missing heritability”. We describe a regulatory network that operates in breast cancer cells (Figure 1). The structure of the network fits well with what is known about key regulators of mammary development and cancer. Within this network we identify five master regulators that are associated with the FGFR2 response. We find that ERα is a key mediator of the FGFR2 response and identify SPDEF as a novel co-regulator of the ERα network. Our approach – described in a paper under review – of identifying an underlying regulatory network that can subsequently be interrogated with gene signatures is applicable to GWAS for many other complex diseases.

Image analysis of breast cancer tissue improves and complements genomic data to predict patient survival
The tumour microenvironment is a complex milieu that includes not only the cancer cells but also the stromal cells, immune cells, and even normal, healthy cells. Molecular analysis of tumour tissue is therefore a challenging task because all this ‘extra’ genomic information can muddle the results. Conversely, biopsy tissue staining can provide a spatial and cellular readout (architecture and content), but it is mostly qualitative information. In response, we have developed a quantitative, computational approach to pathology (Yuan et al, Sci Transl Med 2012; 4: 157ra143). When combined with molecular analyses, we were able to uncover new knowledge about breast tumour biology and, in turn, predict patient survival (Figure 2).

Solid tumours are complex mixtures of cell types, which is rarely taken into account when analyzing molecular profiles of tumour samples, because de-convoluting high-dimensional cancer data is almost impossible without knowing the precise cellular composition of each sample. Additionally, tissue architecture is generally not reflected at all in molecular profiles. We have addressed both problems by leveraging a commonly available, but quantitatively largely neglected, source of information: H&E-stained images of tumour sections. We have shown how histopathological images de-convolute mixed signals in molecular data and identify prognostic features of tumour architecture. In two cohorts of ~300 breast cancers (taken from the METABRIC collections; Curtis et al, Nature 2012; 486: 346), our approach led to profound insights that will advance translational medicine. We developed a novel algorithm to correct copy number data for cellularity and an integrated pathological-genomic
predictor for survival. Most importantly, we found surprising prognostic value in the spatial organization of the tumour tissue architecture.

**Diverse epigenetic strategies interact to control epidermal differentiation**

Experimental perturbations like RNAi are key approaches at the forefront of functional genomics. A goal that is becoming more and more prominent in both experimental as well as in computational research is to leverage gene perturbation screens to the identification of molecular interactions, cellular pathways and regulatory mechanisms. Research focus is shifting from understanding the phenotypes of single proteins to understanding how proteins fulfil their function, what other proteins they interact with and where they act in a pathway. Novel ideas on how to use perturbation screens to uncover cellular wiring diagrams can lead to a better understanding of how cellular networks are deregulated in diseases like cancer.

In collaboration with Klaas Mulder (now at Nijmegen University, The Netherlands) we combined siRNA-based genetic screening and computational approaches to map putative functional interactions among 332 chromatin modifiers in primary human epidermal stem cells. Detailed analysis of a significant sub-network revealed a high degree of true genetic interactions among its components, which physically associated with distinct, yet functionally equivalent gene sets. The functional redundancy in gene expression programs conferred by the epigenetic network we have identified thus protects stem cells from differentiation (Mulder et al, Nat Cell Biol 2012; 14: 753).

This project has led to insights into biology and new methodological developments. We propose to integrate rich phenotypes of multiple single gene perturbations to robustly predict functional modules, which can subsequently be subjected to further experimental investigations such as combinatorial gene silencing (Wang et al, PLoS Comput Biol 2012; 8: e1002566). This addresses an important problem in human functional genomics: Combinatorial gene perturbations provide rich information for a systematic exploration of genetic interactions, but despite successful applications to bacteria and yeast, the scalability of this approach remains a major challenge for higher organisms such as humans. The novel experimental and computational framework we describe efficiently addresses this challenge by limiting the ‘search space’ for important genetic interactions.

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Understanding the roles of proteases in tumour biology

The successful development of tumours is determined by the tissue environment in which the ‘host’ participates in the induction, selection and expansion of the neoplastic cells. Malignant tumour cells recruit vasculature and stroma through the production of stimulatory factors. The locally activated host environment (both cells and extracellular matrix) in turn modifies the proliferative and invasive behaviour of tumour cells. The nature and function of the activating factors involved and the subsequent effectors are important areas of basic biological research in the field of cancer studies. Extracellular proteases are major effectors of both cell-cell and cell-extracellular matrix interactions, modifying ECM integrity, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis. Early data from screens of cancer tissues have shown that different patterns of protease elevation occur and that the relationship of expression to tumour progression and the contribution of individual cell types – tumour cells, fibroblasts, endothelial cells and inflammatory cells – requires detailed dissection. A major aim of the drive to understand protease biology within a specific tumour environment relates to the need to assess potential targets within the interface between tumour cells and the ‘host’ cells that may be appropriate for therapeutic intervention. It is anticipated that the understanding and the manipulation of protease function will give clear leads as to the critical stages in the breakdown of the normal tissue-cell ‘society’ that occurs in neoplasia.

Within this remit our research is focussed on cell surface associated forms of the zinc-dependent proteases, notably the membrane type-1 matrix metalloproteinase (MT1 MMP; MMP14) and members of the disintegrin metalloproteinases (ADAM17). We aim to elucidate how these metalloproteases function in the regulation of extrinsic effectors at the cellular and molecular level, as well as proceeding to complex tumour models addressing tumour-stromal interactions. Based on our findings we are developing and evaluating novel approaches to understand the regulation of metalloproteases in tumour systems.

Membrane associated metalloproteases

The membrane type 1 matrix metalloproteinase MT1 MMP plays a major role in tumourigenesis, including tumour cell migration, aspects of stromal cell function and angiogenesis. We are collaboratively developing novel inhibitors of MT1 MMP. In particular, the characterisation of scFv antibodies that we have isolated is being used to address the question of the importance of exosite interactions in the collagenolytic capacity...
of MT1 MMP. The effect of enzyme inhibition in cell model systems, such as the ‘mini tumour’ (see below) have demonstrated the key role of this enzyme.

The disintegrin metalloproteinases are also regulators of cellular signalling and we are studying ADAM17 in this respect. We are particularly interested in the generation of soluble EGF receptor ligands and the activation of HER receptors which occur downstream of the activation of a wide variety of other receptor types. The significance of ADAM17 activity is being investigated in cell models using shRNA and siRNA techniques and novel antibody tools that we have recently developed. ADAM regulation by G-protein coupled receptors and redox mechanisms are being investigated. The roles of ADAM17 in the development of ovarian, breast, colorectal and head and neck cancers are being evaluated collaboratively using gene ablation or over-expression studies in animal models.

Biochemical studies have focussed on the structure-function relationships of ADAM17 and projects are in progress on the role of different ADAM domains in the proteolytic ‘shedding’ of cell surface proteins. Notably, using phage display technology we have developed highly specific inhibitory single chain fragment antibodies to the extra-catalytic cysteine rich domain. These have been refined to generate ‘cross-domain’ antibodies that also bind the catalytic domain (Figure 1). The most powerful antibody is being evaluated in cell and in vivo models of tumourigenesis.

**3D in vitro models**

In order to carry out molecular studies and inhibitor screens on the complexity of cells within tumour tissue, we have set up several more complex 3D models of interacting cells in collagen gels. In particular we have developed a novel *in vitro* ‘mini-tumour’ angiogenesis model, by co-culturing cancer cell lines with primary human endothelial cells and fibroblasts. All the cell types are in direct contact and in a three dimensional system, in which pre-capillary sprout formation can be easily quantified. This spheroid model has been shown to promote the development of pre-capillary sprouts, under the influence of the tumour cells and independent of external growth factors. Sprout formation was shown to respond to known anti-angiogenic compounds and growth factor receptor inhibitors similar to observations made in clinical trials. Our major goal is to evaluate metalloproteinase function in individual cell types and the outcomes of their abrogation. The use of function blocking antibodies and siRNA or shRNA for our protease targets has been evaluated. We can specifically target fibroblasts prior to their inclusion in the model and examine their role in supporting pre-capillary sprout formation. We have shown that fibroblast MT1 MMP and ADAM10 expression are key to angiogenic sprout formation in the model and we are currently investigating their roles in the mechanisms of fibroblast regulation of endothelial cell behaviour. Illumina microarray analyses have indicated a number of genes that may be acting as a link between fibroblast MT1 MMP and endothelial cell activity in co-cultures.

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DNA methylation, post translational histone modifications, together with chromatin structure, underpin the epigenetic organization of the genome which responds to the environment and changes during tissue differentiation, cell cycle, cell death, wound healing and neoplasia.

Genomic imprinting is a fascinating epigenetic process that marks the gametic origin of a subset of genes and results in the expression of one parental allele and the reciprocal silencing of its homologue. Imprinted genes are exceptionally stable in their maintenance of DNA methylation (Woodfine et al., *Epigenetics Chromatin* 2011; 4: 1), despite being expressed in a tissue specific manner and resist DNA methylation reprogramming during normal development. This makes them tractable markers for detecting abnormal epigenetic reprogramming in cancer.

Imprinted genes are well characterised in terms of their epigenetic marks and expression profiles and are therefore an excellent model system in which to study the impact of DNA methylation and chromatin changes in cancer. Aberrant imprinting of the insulin-like growth factor 2 (*IGF2*) gene locus is part of the aetiology of congenital growth disorders such as Beckwith Wiedemann syndrome (BWS, OMIM#130650), as well as various human cancers including Wilms’ tumour, rhabdomyosarcoma, hepatoblastoma, colorectal and breast carcinomas (Cooper et al., *Eur J Hum Genet* 2009; 13: 1025; Murrell, *ScientificWorldJournal* 2006; 6: 1888). Imprinting of *IGF2* and *H19* are regulated by an insulator element upstream of *H19*. The zinc finger protein CTCF binds to the insulator and mediates its function, such that when CTCF is bound on the unmethylated maternal allele, the maternal copy of *IGF2* cannot access enhancers downstream of the *H19* gene (Bell and Felsenfeld, *Nature* 2000; 405: 482). Methylation at the insulator sequence on the paternal allele prevents CTCF binding, inactivates the insulator function and enables the *IGF2* gene to access the enhancers.

We have previously shown that in mice chromatin looping is mediated by CTCF binding at the insulator (Murrell et al., *Nat Genet* 2004; 36: 889), and have hypothesised that DNA methylation of the insulator sequence would result in differential loops on the maternal and paternal allele. Subsequently, genome-wide studies have shown that cohesin complexes co-localise onto the same DNA sequences as CTCF (Wendt et al., *Nature* 2008; 451: 796). We therefore speculated that cohesin may have a function in holding chromatin loops together by connecting two DNA molecules in cis, in an analogous manner to its role in holding two sister chromatids together. We found that CTCF sites upstream, within and downstream of the locus interacted to form looping domains. Cohesin depletion significantly reduced the interaction frequencies between CTCF binding sites suggesting that cohesin is required for stabilisation of chromatin loops. Interestingly when chromatin looping conformation was destabilised *IGF2* expression was up-regulated and biallelically expressed, despite methylation at the insulator sequence not changing, indicating that chromatin conformation changes can override epigenetic imprinted information (Nativio et al., *PLoS Genet* 2009; 5: e1000739; Nativio et al., *Hum Mol Genet* 2011; 20: 1363).

Others have confirmed that cancer cells have changes in chromatin conformation at the *IGF2-H19* locus (Vu et al., *Hum Mol Genet* 2010; 19: 901). We previously showed a disconnection between DNA methylation at the *IGF2* gene (the DMR0 region) and the *H19* insulator in Wilms’ tumours (Murrell et al., *PLoS ONE* 2008; 3: e1849), colorectal and breast cancer (Ito et al., *Hum Mol Genet* 2008; 17: 2366). In colorectal cancer hypomethylation of the *IGF2* DMR0 is prevalent as an early event and may potentially even be indicative of cancer (Ito et al., *Hum Mol Genet* 2008; 17: 2633; Ibrahim et al., *Gut* 2011; 60: 499).

Methylated cytosines (5mC) have recently been shown to be a substrate for hydroxylases that convert them to 5-hydroxymethylcytosine (5hmC) (Tahiliani et al., *Science* 2009; 324: 930; Ito et al., *Science* 2011; 333: 1300) and it is widely postulated that 5hmC may be an intermediate molecule during DNA demethylation. Since global hypomethylation is a feature of several cancers, we decided to look at 5hmC levels in a set of well defined colorectal cancers consisting of matched normal tissue hypoplastic polyps, adenomas and also tumours (Figure 1). We found
Figure 1
Global reduction of 5-hydroxymethylcytidine (5-hmC) in colon cancer associated with rapid cell proliferation. (A) Cycle of covalent modifications to cytidine at the 5 C position. DNA methyltransferases add a methyl group to form 5-mC which can be oxidised by TET or ELP enzymes to form 5-hmC. Hydroxymethylated cytidine may be further oxidised to formyl and carboxyl cytidine (5-fC and 5-cC) and finally repaired into an unmethylated cytidine. 5-mC and 5-hmC are also deaminated and replaced into unmethylated cytidine by DNA repair. (B) Global 5hmC levels measured on total DNA extracted from colon samples at sites away from a tumour (normal away, NA), adjacent to the tumour (normal close, NC), adenoma (Ad) and tumour (T) in two colorectal cancer patients. The left-hand panel is a DNA stain indicating DNA loading, the middle panel is immunoblot probed with an antibody for hydroxymethylated DNA (hmC), and the right-hand panel is the same blot probed with an antibody to methylated DNA (mC). (C) Spatial distribution of 5hmC in normal mouse intestine – 5hmC is strongly present in terminally differentiated epithelia (long arrow) and weakly present in dividing transit amplifying cells (short arrow).

that 5hmC levels are strikingly reduced at early stage of carcinogenesis such as in adenomas, but also in some hypoplastic polyps. Our results further indicate that genes marked by 5hmC are actively transcribed and protected from DNA methylation changes in tumours. It is now beginning to look as if 5hmC plays a tissue specific role in gene regulation rather than simply being an intermediate of DNA demethylation and we are now actively looking for the function and associated mechanism of this newest epigenetic 5hmC mark.

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Cellular senescence is a state of stable cell cycle arrest with active metabolism. Similar to apoptosis, senescence can be a failsafe program against a variety of cellular insults. In contrast to apoptosis, in which cytotoxic signals converge to a common mechanism, senescence is typically a delayed stress response involving multiple effector mechanisms.

These effector mechanisms include epigenetic regulation, the DNA damage response, the senescence-associated secretion phenotype (SASP) and autophagy. The relative contribution of these effectors varies depending on the trigger and cell type, and it is possible that the combination and balance of these effectors determines the quality of the senescence phenotype. Thus, to understand the senescence program, it is important to identify new effector mechanisms and examine how they associate with each other, and also to identify which effector mechanisms could be potential targets for cancer therapy.

**Genome-wide analysis of heterochromatin components in SAHF**

Certain types of cells undergo distinct alterations in chromatin structure during senescence, called senescence-associated heterochromatic foci (SAHF). SAHF have been widely used as a marker of senescence, and more importantly, several new components of senescence machinery have been successfully identified using SAHF as a readout. Thus, it is important to understand SAHF structure in more detail and how SAHF are actually formed. Using the oncogenic Ras-induced senescence model in normal human diploid fibroblasts (HDFs), we have shown that SAHF are indistinguishable from the inactive X (Xi) chromosome, one of the best studied heterochromatin models, and other groups recently suggested that each individual SAHF might represent each chromosome territory. In contrast to Xi, SAHF formation can be dynamically regulated in HDFs, thus providing a unique tool to study not only senescence, but also chromatin biology. To further characterise SAHF in detail, we have investigated a dynamic redistribution of the specific histone modifications using confocal microscopy as well as chromatin-IP coupled with deep sequencing (ChIP-seq). In collaboration with the Tavaré group, we have shown that SAHF formation results in a concentric chromatin architecture, not only segregating the chromatin of individual chromosomes into heterochromatin and euchromatin, but also concentrating histones H3K9me3 and H3K27me3 (markers of constitutive and facultative heterochromatin, respectively) in non-overlapping layers within SAHFs. Surprisingly, the linear epigenomic profiles of these repressive marks are highly static despite the dramatic chromatin structure alteration. This is in marked contrast to the spreading of repressive marks occurring during embryonic stem cell differentiation. Our data indicate that the high-order chromatin structure change during SAHF formation is achieved mainly through the spatial rearrangement of pre-existing heterochromatin, rather than spreading of heterochromatin (Chandra et al, *Mol Cell* 2012; 47: 203)(Figure 1). We are currently characterising ‘SAHF-modulating’ factors and their relationship with individual gene regulation during senescence.

**TOR-autophagy spatial coupling compartment, TASCC**

Oncogene induced senescence (OIS) is a very dynamic process where cells typically undergo an initial burst of cell proliferation (‘mitotic phase’), followed by the induction of pro-senescent factors (‘transition phase’). Eventually, the senescent phenotype dominates (‘senescence phase’). During the transition phase, oncogenic and pro-senescent activities work against each other, and senescence usually prevails in normal cells. How cells can achieve such a drastic phenotypic remodelling is unclear. We are interested in another layer of gene expression control, namely protein metabolism, during senescence. We reason that global epigenetic alteration should be coupled with efficient protein turnover as a part of the execution of epigenetic ‘blue prints’, in such an emergent context. Consistent with this idea, we have identified that autophagy, a bulk protein degradation program, facilitates synthesis of IL6/8, which are central components of SASP (Young et al, *Genes Dev* 2009; 23: 798).

More recently, we have shown that mTOR and autophagy cooperatively facilitate SASP through forming a new cellular compartment, the TOR-autophagy spatial coupling compartment (TASCC), which provides a local environment enriched for...
amino acids and mRNA translation machinery (Narita et al, Science 2011; 332: 966). Autophagic degradation and mRNA translation are regulated in opposite directions by mTOR, the central regulator of protein synthesis. Using immunofluorescence, we identified cytoplasmic compartments, which are enriched for both autolysosomes (the end stage of autophagy) and mTOR. The TASCC invariably localises in the vicinity of the rER-Golgi apparatus, where lysosomal/membrane and secretory proteins are synthesised and processed. Thus, it is conceivable that the concentrated localisation of mTOR on autolysosomes facilitates localised mRNA translation, including lysosomal proteins (thus reinforcing TASCC formation) as well as SASP/SMS components. Notably, since mTOR inhibits the initial stage of autophagosome formation, the compartmentalisation of mTOR with autolysosomes permits autophagosome formation outside the TASCC. Therefore, the TASCC would allow a simultaneous activation of anabolic (by mTOR) and catabolic (by autophagy) processes. In addition, we have shown that a dominant negative mutant of RagB GTPase inhibited mTOR recruitment to the TASCC, resulting in decreased synthesis of IL6 and IL8 during Ras-induced senescence. This is consistent with seminal work from David Sabatini’s group (Whitehead Institute), showing that mTOR can be recruited to the lysosomal surface in response to amino acids, in a Rag GTPase-dependent manner. We have also shown that a TASSC-like structure is not limited to Ras-induced senescence, and we are currently investigating an implication of this structure more generally in the cancer context.

Identification of senescence-associated p53 function (in collaboration with the Tavaré group) A tumour suppressive transcription factor, p53, plays a critical role in many stress responsive phenotypes, including DNA damage checkpoints, apoptosis, and senescence (Figure 2). Although ample data have supported a role for p53 in senescence, the precise mechanism or ‘senescence-specific p53-targets’ are not known. To address this issue, we are currently using HDFs, where we can induce different phenotypes, in which p53 plays a crucial role, depending on environmental stimuli or other conditions. These phenotypes include, senescence, apoptosis, and acute DNA damage response. Using expression microarrays in conjunction with stable RNAi technology as well as p53 ChIP-seq, this system would allow us to understand both general and phenotype-specific p53 functions.

Figure 1
Multi-layered structure of SAHFs in Ras-induced senescence (RIS) IMR90 cells. Confocal images of immunofluorescence for histone H3K9me3 (a marker of constitutive heterochromatin), H3K27me3 (a marker of facultative heterochromatin), and H3K36me3 (a marker of transcription elongation) in the cells shown. From Chandra et al, Mol Cell 2012; 47; 203.

Figure 2
Model system for understanding a comprehensive picture of p53 functions.

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We are a surgical translational research group with a focus on hormone relapsed prostate cancer.

Our clinical practice informs and underpins the research. Biorepositories from our robotic prostatectomy programme and from ProteCT (the largest ever surgical randomised controlled trial in prostate cancer), have led to important collaborative research with Doug Easton at the Strangeways Research Laboratory and Ros Eeles at the Institute of Cancer Research (ICR) (Al Olama et al, *Hum Mol Genet* 2013; 22: 408). We have a funded collaboration with Ros Eeles, Colin Cooper (ICR), Doug Easton, and Mike Stratton (Sanger Institute) to carry out next generation sequencing of prostate cancers and our first paper is in preparation. We have appointed Dr Charlie Massie to a new computational biology post.

Main discoveries

1. The AR and hormone relapsed prostate cancer (HRPC)

Androgen receptor (AR) signalling is maintained in most men with castration-independent prostate cancer and new management and therapeutic approaches are needed. Our goals are to identify and characterise markers that better predict progression, and to identify signalling pathways that lead to more effective treatments. The AR remains the primary target for treatment and the rationale remains strong for better targeting of this pathway and to uncover biomarkers. We are working with human tissue wherever possible, but our portfolio now includes pre-clinical *in vivo* models, which will give us better information on how individual genes function throughout tumour development. Examples include p53/pRb or PTEN prostate specific knockouts, which express the luciferase gene in tumour cells and makes them traceable through bioluminescence imaging.

We have performed AR ChIP-seq in fresh tissue samples obtained from men undergoing prostate surgery to study the transcriptional activity of the AR in a variety of patients with different stages of disease. This includes those with early, localised disease and in particular those with advanced castrate-resistant prostate cancer (CRPC). In contrast to previously published studies using cultured cells, this study of fresh human tissue has shown for the first time how other prognostic transcription factors (including STAT, E2F and MYC) might cross-talk with the AR in clinical samples, based on recruitment to DNA. We have found that the AR is repositioned in CRPC (Figure 1), including thousands of tissue-specific binding sites. Functional studies supported a model of altered signalling *in vivo* directing AR binding, with specific implications for our understanding and management of CRPC. We identified a 16-gene signature which outperformed a larger *in vitro*-derived signature in clinical datasets, showing the importance of persistent AR signalling in CRPC and revealing potential targets which would otherwise not have been implicated in CRPC. As therapies are currently being developed to target c-MYC and STAT, this study may prompt additional combination trials whilst offering possible surrogate response markers (Sharma et al, *Cancer Cell* 2013; 23: 35).

Furthermore, this demonstration of the critical role of cellular context in the regulation of transcription factor target selection and gene regulation highlights a wider need to utilise clinical material for the study of oncogenic transcription factors. Another study is underway studying the role of two novel tyrosine kinases in advanced prostate cancer as well as determining the impact of the AR in the DNA damage response.

2. Studies on HES6

We have now completed our work on HES6, which is a transcription co-factor best known for its role in fate decisions of certain stem cell lineages. Its expression is increased by c-Myc and the AR, and this creates an altered transcriptional environment where prostate cancer cell division and growth is maintained in the presence of an active AR but in the absence of ligand binding by dihydrotestosterone. We have shown that Hes6 is able, in isolation, to drive cell growth in an androgen deprived/castrate setting, and that this maintained proliferation occurs in the context of a transcriptionally active AR. We have also shown that cell cycle and metabolic networks are activated, including up-regulation of E2F family members, CDC2, UBE2C, CDC20, Aurora kinases, PLK1, Cyclins, AMACR, GDF15 and LDHA. We have shown by ChIPseq the cooperation between Hes6, E2F1 and the AR to maintain G1/S transition and cell proliferation. This publication is currently under revision.
3. Studies on biomarkers
We have identified three new biomarkers and are continuing to study MSMB in several thousands of men in collaboration with Henrik Gronberg at the Karolinska Institute. In addition to looking at proteins we have also identified an RNA signature found in circulating blood that can identify patients with aggressive forms of cancer (Sharma et al, Cancer Cell 2013; 23: 35).

4. Studies on beta-arrestin1 (ARRB1)
ARRB1 plays a role in cancer progression and some tumours show elevated levels in the nucleus where it may regulate gene expression via epigenetic mechanisms. We have shown that ARRB1 contributes to a metabolic shift to aerobic glycolysis via regulation of HIF1A activity through regulation of SDHA and FH expression in prostate cancer cells. This is the first example of an endocytic adaptor protein regulating metabolic pathways and implicates ARRB1 as a potential tumour promoter. A paper is in revision.

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The control and evolution of tissue-specific gene expression

The proteins that control DNA, known as transcription factors, bind to it in a combinatorial manner in yeast and bacteria, and our earliest work showed that this combinatorial binding occurs in mammalian tissues as well. Master regulators in primary human hepatocytes form a highly interconnected core circuitry that frequently bind promoter regions in clusters, particularly at highly regulated and transcribed genes (Odom et al, *Mol Syst Biol* 2006; 2: 2006.0017). We have recently found that transcriptional regulation diverges very rapidly in mammals (Schmidt et al, *Science* 2010; 328: 1036; Odom et al, *Nat Genet* 2007; 39: 730). Despite this evolution, we found specific genetic architectures that appear to preserve a small handful of transcription factor binding events across large evolutionary timescales (>300 million years) (Schmidt et al, *Science* 2010; 328: 1036).

In asking why rapid variation occurs among most transcription factor binding events, we realised that a number of causative factors could contribute. These possible causes may be the result of variability of genetic sequences, the types and number of marks left in the histone proteins that package DNA (commonly thought of as an epigenetic code), or even diet or environmental differences between different species. In order to isolate a single one of these variables, we used a previously created mouse model of Down’s syndrome that carries a virtually complete copy of a human chromosome (O’Doherty et al, *Science* 2005; 309: 2033). By exploiting this aneuploid mouse strain, a unique and powerful genetic tool designed for an entirely different purpose, we determined that genetic sequence dominates other factors in directing transcription (Wilson et al, *Science* 2008; 322: 434). More recently, we have used this mouse to investigate how human-specific repetitive elements contain latent regulatory potential that is unmasked in a mouse heterologous environment (Ward et al, *Mol Cell* 2013; 49: 262).

The origin, regulation, and evolution of RNA transcription

We have been using similar comparative functional genomics approaches to look at the regions of the genome that are transcribed, but which do not code for proteins. These regions are known as non-coding RNAs, and range from well-characterised species like tRNAs and rRNAs to newer categories of regulatory nucleic acids like microRNAs, piRNAs, and endogenously expressed RNAi. We recently published results describing previously unseen functional conservation in tRNA gene transcription driven by RNA polymerase III, that only becomes apparent after analysis of data from multiple mammalian species (Figure 1) (Kutter et al, *Nat Genet* 2011; 43: 948). We have recently reported that the rapid evolution of long noncoding RNAs between closely related mammals appears to be a mechanism to alter nearby gene expression (Kutter et al, *PLoS Genet* 2012; 8: e1002841). Finally, by using closely related, but
Figure 2
CTCF binding evolution across mammals reveals new mechanisms of genome evolution, driven by repetitive elements.

still interbreeding species of mice, we were able to dissect the relative cis- and trans- contributions to gene expression, discovering that in mammals, compensatory cis and trans effects appear to be the rule during evolution (Goncalves et al, Genome Res 2012; 22: 2376). This was an intriguing finding because other systems studied to date, such as flies and yeast, have found much stronger trans contributions at close evolutionary distances.

The complex interplay of CTCF, cohesin, and repetitive sequences in the genome
The CTCF protein is a genomic anchor that appears to have roles in regulating mitosis and meiosis, and in insulating chromatin and gene expression across the genome. Many of its functions are mediated by the cohesin complex in mammalian cells. We have discovered how the cohesin complex can co-regulate gene expression with tissue-specific transcription factors in the absence of its canonical partner CTCF (Schmidt et al, Genome Res 2010; 20: 578). By creating large, high-resolution maps of cohesin and tissue-specific transcription factor binding in mouse liver cells, we revealed that cohesin appears to stabilize large complexes of proteins, thus reducing the required motif quality for transcription factor binding (Faure et al, Genome Res 2012; 22: 2163). We have also explored how most lineage-specific CTCF binding is not ‘born’ in the same way as other tissue-specific transcription factors, but appears in the genome via carriage within repetitive elements that are active in a species-specific manner in mammals (Schmidt et al, Cell 2012; 148: 335) (Figure 2).

Collectively we found that these newborn CTCF binding events are as functionally active as ancient ones found in six or more mammals, and that these ancient binding events show fossilized remains of the prior repeat expansions that gave birth to them.

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Our research focuses on inherited susceptibility to breast and other common cancers. Our overall aims are to identify the genes involved and their mechanisms, so as (1) to define high-risk groups within the population, and (2) ultimately, to devise strategies for prevention based on the mechanisms of risk.

To date our work has been following up the results of the genome-wide association studies in breast cancer that we and our colleagues initiated. The chance of an individual developing breast cancer is roughly two-fold greater if that individual has a close relative with breast cancer. Twin studies indicate that this risk is largely genetic. The genes that confer this risk have been sought either by genetic linkage mapping in multiple-case families, or by genome-wide association studies (GWAS). The former have identified rare but higher risk alleles such as those of BRCA1 and BRCA2, while GWAS have identified common variants that each carry only a small risk of cancer. BRCA1 and 2 explain about 15–20% of the estimated total genetic risk of breast cancer, and the 50 or so loci so far identified through GWAS a further 14%.

One question is how to find the genes that account for the ‘missing’ 70% or so of heritability. Larger and more powerful GWAS have already been initiated and are expected to find additional common risk variants, while genome resequencing will identify an unknown contribution from rare genetic variants. Pending these studies we are investigating other approaches to inform on cancer susceptibility in breast and in lung cancer.

Individually the common, low risk variants have only small effects and cancer GWAS have been criticised for the lack of novel biological insights gained from the identification of these variants. To address this we have taken a systems biology approach to study the architecture of the gene networks in breast cancer that are associated with FGFR2, the top GWAS ‘hit’. By combining genomic and computational approaches we have found that risk SNPs, including those that do not reach genome-wide significance but lie at the top end of the risk distribution, map preferentially near FGFR2 responsive genes, indicating that risk genes cluster in pathways. Furthermore our findings suggest that at least some of the variants at these loci are functional and contribute to the “missing heritability”. Our work also describes a regulatory network that operates in breast cancer cells and identifies those parts of the network that are associated with FGFR2 signalling (Figure 1). The structure of the network fits well with what is known about key regulators of mammary development and cancer. Within this network we identify five master regulators that are associated with the FGFR2 response. We find that ERα is a key mediator of the FGFR2 response and identify SPDEF as a novel co-regulator of the ERα network. In the future we aim to extend our network studies to investigate differences in the regulatory network between the distinct subclasses of breast cancer.

We have also carried out functional analyses of individual risk loci with the aim of identifying functional variants and defining their target genes. We have focussed our functional analysis on genomic regions that confer risk to multiple different types of cancer, such as the risk region on chromosome 11q13, which confers risk of both breast and renal cancer. In collaboration with the Strangeways Research Laboratory and the Queensland Institute for Medical Research we have investigated this locus by a combination of fine mapping, analysis of chromosome architecture, transcription assays and biochemical analysis of protein-DNA interactions. Our results indicate that risk is mediated by the interplay of three independent risk SNPs within the same genomic interval. We have identified the transcription factors ELK4 and GATA3 as mediators of risk and showed that the risk SNPs reduce the transcriptional activation of long range enhancers. Chromatin conformation capture suggests that the target gene is cyclin D1 (French et al. Am J Hum Genet 2013; In press). We find that the causative variant confers almost two-fold greater risk than was first calculated for the tagging SNP at this locus. The functional analysis of individual risk loci is now being followed up with genome-wide studies of allele-specific binding of RNA polymerase II to obtain a more direct read-out of transcriptional activity.
In lung cancer, less than 5% of the estimated genetic variance of risk is explained by the DNA sequence variants so far identified. We are taking two approaches to search for the ‘missing heritability’ in this cancer. In one approach we will attempt to use novel assays of DNA repair capacity to ask whether differences in DNA repair affect individual risk of lung cancer in smokers. In the second approach we will ask a more open-ended question about whether there are individual differences in the response of airway cells to cigarette smoke injury that correlate with lung cancer. The read out of injury response will be patterns of mRNA and miRNA expression. If differences are found these may provide useful markers of risk; if the patterns of expression that are associated with risk can be resolved into networks that indicate mechanism, this may provide possible targets for mechanism-based prevention. Furthermore we are investigating whether expression changes in nasal epithelium, which is more accessible to sampling than the bronchial airways, can provide a surrogate read-out to changes occurring in the bronchial epithelium and may thereby help to identify individuals at increased risk of developing lung cancer.

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Blood plasma of cancer patients contains DNA fragments that originate from the tumour. We leverage advanced genomic technologies to extract information from these fragments, using them as a “liquid biopsy” for non-invasive diagnostics.

Cancer is a disease of the genome, characterized by and caused by variable patterns of genomic alterations. Cancer is difficult to treat because every cancer is different, and can further evolve over time and in response to treatment. Current methods for monitoring cancer dynamics are limited: protein markers and imaging estimate tumour burden, but can’t assess genomic status. Biopsies give a snapshot of genomic changes, but can’t be used repeatedly. Better methods to study tumour evolution can promote research and greatly improve cancer care.

Noninvasive diagnostic tools for cancer using circulating DNA

We employ emerging molecular technologies to develop new diagnostic approaches. Our focus is on circulating tumour DNA (ctDNA) as a noninvasive modality to assess evolution of solid malignancies. This is DNA originating from cancer cells, carrying tumour-specific genomic alterations, that is present as short cell-free fragments in body fluids such as blood plasma. ctDNA can be collected noninvasively via blood samples and has the potential to be immensely informative.

The field of prenatal diagnostics is revolutionised by non-invasive tests that assay fetal DNA fragments in maternal plasma. Parallel progress in cancer has been lagging, because genomic loci of interest are not well defined, and levels of tumour DNA in plasma are variable and generally lower: 2 ml of plasma may contain as many as 10,000 copies of DNA from healthy cells but only a few dozen copies of the tumour genome. The mechanisms through which tumour DNA reaches blood circulation are unclear, although fragmentation patterns of DNA in the plasma suggest it may originate from cell death. Overall levels of circulating DNA are higher in cancer patients compared with healthy controls, but these differences are not consistent enough for robust diagnostic tools. Maturation of genomic technologies empowers a different approach that focuses on those fragments that carry cancer mutations.

We use a combination of molecular methods such as next-generation sequencing and digital PCR, and develop bespoke data analysis algorithms that allow such data to be used for sensitive measurement of rare alleles (Figure 1). We apply these methods to monitor mutation status and circulating tumour DNA levels in serial samples collected from patients during treatment and follow-up, in close collaboration with clinical and translational research groups. High levels of circulating tumour DNA in cancer patients are a bad prognostic indicator. Changes in circulating tumour DNA levels may indicate response to therapy or disease progression, and indeed may prove to be the earliest indicator of changes to tumour burden.

We have recently shown that circulating tumour DNA can be used as a “liquid biopsy” to analyse cancer mutations in a non-invasive way (Forshew et al, Sci Transl Med 2012; 4: 136ra68) (Figure 2). This means that a blood test may reduce the dependence on obtaining a tumour biopsy, which involves invasive procedures. In certain clinical scenarios, for example where obtaining a biopsy is not possible, analysis of a blood samples using techniques such as those we are developing may yield important diagnostic information. Such analysis can also be done more frequently, and in the future may be incorporated as part of a clinical routine analysis.

At present, a significant effort in cancer research is in understanding the roles of tumour heterogeneity and evolution on drug response and resistance. Much of this depends to date on collection of multiple biopsy samples from generous patients, which limits its applicability and adds complexity and costs. Methods we are developing can provide data in a noninvasive manner and can greatly enhance the pace of research.

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Figure 1
Workflow for studies on circulating tumour-specific DNA. DNA obtained from a patient’s tumour or biopsy sample is used to identify tumour-specific genomic alterations. Assays are designed to specifically measure these tumour-specific DNA sequences. Assays are validated using tumour DNA as positive control and DNA from other subjects (and normal) as negative controls. The assays are used to measure ctDNA levels in blood samples from the same patient. These data are compared to clinical information to study ctDNA dynamics and diagnostic potential.

Figure 2
This patient presented with advanced ovarian cancer, and was treated by debulking surgery and chemotherapy. Sequencing of an initial biopsy sample (from the right ovary) identified a mutation in TP53, a known tumour-suppressor gene. Fifteen months after surgery, the patient relapsed. Analysing DNA from a plasma sample, a method we developed called TAm-Seq (for Tagged-Amplicon deep Sequencing) identified an unexpected mutation in EGFR, a known oncogene which can be inhibited by targeted therapies. The same mutation was present in an additional sample collected ten months later. Retrospective analysis of multiple samples removed at the time of initial surgery showed than the mutation in EGFR was present as a minor sub-clone in tumour masses from the omentum (adapted from Forshew et al, Sci Transl Med 2012; 4: 136ra68).
My laboratory currently has four main research themes:

1. Determining the cells of origin of different types of breast cancer

   Breast cancer is a heterogeneous disease with at least five molecular subtypes and 18 histological subtypes identified. Our laboratory is interested in elucidating the mechanisms that account for this heterogeneity. One possible mechanism is that different types of breast cancers initiate in, and are propagated by, different types of mammary cells. We have recently identified two novel types of progenitor cells within the human mammary epithelium and we are currently investigating how they relate to breast tumours. We are doing this by using engineered viruses to introduce common oncogenic mutations into different cellular backgrounds to see if these specific mutations preferentially exert their effects in specific types of cells. In addition, we are particularly interested in finding out if these mutations can impart some properties of stem cells to committed progenitor and differentiated cells.

2. Characterisation of the stem cell properties of mammary myoepithelial cells

   Myoepithelial cells are contractile muscle-like cells in the mammary gland that function to squeeze milk out of the gland during lactation. Because of their specialized function, and because these cells are rarely observed to be undergoing cell division, these cells are perceived to be a mature cell type with little proliferative capacity. However, we have made the curious observation that these cells, when placed into a novel cell culture system that we have developed, quickly revert to a mammary stem cell state (Figure 1). Thus we are currently investigating our hypothesis that mammary myoepithelial cells also function as a quiescent mammary gland stem cell.

3. Characterisation of human ovarian cancer stem cells (collaborative project with James Brenton, CI)

   Serous ovarian cancer is an aggressive disease that initially responds to chemotherapy, but approximately 70% of patients will relapse and become resistant to therapy. It is our hypothesis that this resistance is mediated by the emergence of a sub-population of ovarian cancer stem cells. We are currently evaluating the proliferative potential of phenotypically distinct subsets of ovarian tumour cells in order to identify the putative cancer stem cells. Future research includes tracking experiments to follow the fate of individual clones during chemotherapy and gene expression profiling of these cells.

4. Characterisation of the normal and malignant prostate epithelial cell hierarchy (collaborative project with David Neal, CI)

   We are applying our expertise in the characterisation of the mammary epithelial cell hierarchy to the human prostate epithelial cell hierarchy. We are particularly interested in characterising progenitor cells within the human prostate and their developmental relationships.

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Mammary myoepithelial cells rapidly revert to an undifferentiated state when placed into a novel culture system that we have developed. This image shows a seven day-old colony derived from a single myoepithelial cell. The myoepithelial cell has proliferated to generate a cluster of keratin 5-expressing daughter cells. The keratin 5 is detected by the green staining. Some of these daughter cells will be mammary gland stem cells.
Our work has continued its focus on three main areas: Statistical methods for the analysis of next-generation sequencing data, evolutionary approaches to cancer and methods for the analysis of genomics data.

We are continuing our collaboration with the International Cancer Genome Consortium projects on oesophageal adenocarcinoma and prostate cancer. The former is led by Dr Rebecca Fitzgerald (MRC Cancer Cell Unit), the latter co-led by Professor David Neal (CI). These projects, funded by Cancer Research UK, are sequencing many tumour-normal samples, and should provide interesting and medically relevant information about the aberrations that occur in the genomes of these cancers. Our pilot studies have already highlighted the complexities of large-scale sequencing projects, particularly with respect to the identification of structural variants, SNPs and SNVs, and the computational infrastructure required to support such projects. A number of novel statistical problems arise in the analysis, particularly when dealing with multiple samples from the same patient. The Bioinformatics core facility led by Dr Matt Eldridge in the CI and members of the Wellcome Trust Sanger Institute collaborate with us in this work.

We have continued our collaboration with the Caldas laboratory on the statistical analysis of high-density microarrays from the METABRIC project that has assayed germline and somatic copy number variants, and their impact on expression variation, in some 2,000 breast tumours. A novel classification of breast tumours was recently published in *Nature* (Curtis et al, *Nature* 2012; 486: 346).

Illumina BeadArray technologies continue to be an important tool in cancer studies (such as METABRIC and the ICGC) and we, in collaboration with Mark Dunning (Bioinformatics core) and Matt Ritchie (WEHI), continue to update and support the beadarray Bioconductor package in order to facilitate transparent and flexible statistical analyses of full bead-level data. We have developed over 20 software packages, and our group is committed to providing open source computational tools. We have a number of other ongoing collaborations within the CI, in particular with the Narita and Winton labs. We have also been collaborating with Dr Colin Watts’ lab in Clinical Neurosciences, Dr Christina Curtis at USC and Dr John Marioni at EBI in a study of intra-tumour heterogeneity in glioblastoma.

We have continued our research in the area of evolutionary methods in cancer biology, focussing in particular on spatial stochastic models for the evolution of colorectal cancer. Such models can be used, *inter alia*, to study the cancer stem cell (CSC) hypothesis by comparing the dynamics of molecular markers in a CSC-driven tumour with that of a non-hierarchical growth model. This approach allows us to estimate the CSC fraction in a tumour, and to predict the effects of treatment. We have used high-throughput 454 sequencing to generate large amounts of data on heterogeneity in colon cancer. We have also continued our development of approximate Bayesian computation (ABC) for inference in agent-based models such as those used for colon cancer.

The lab has several new recruits this year. Charlotte Anderson joined the ICGC group as a computer officer. Malvina Josephidou joined as a PhD student, having completed her MEng with Distinction in the Department of Engineering. Mary Fortune completed a summer internship before starting her PhD in the Wellcome Trust Mathematical Genomics and Medicine programme, and Dr Alexandra Jauhiainen visited again from the Karolinska Institute in Stockholm.

Dr Nick Marko completed his Van Wagenen Fellowship supported by the American Association of Neurological Surgeons, and has moved to the MD Anderson Cancer Center in Houston. Dr Ernest Turro left the group to take up a position in the Department of Haematology in the University. Andrea Sottoriva completed his PhD, and is now a postdoc in Dr Christina Curtis’ lab at the Keck School of Medicine of USC.

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Methods generated in the group allow us to detect localized shifts in image registration between the sequencing of the first and second reads of a paired end Illumina run (top panel). Since the spacing of sequence clusters on the flow-cell is stochastic, the shift may cause the second read of one cluster to be read from a different cluster (bottom panel). This can give the appearance of a DNA fragment spanning two chromosomes, and lead to the investigation of spurious chromosomal rearrangements and gene fusions, or at best can mask true rearrangements. We are publishing tools to identify and correct this phenomenon.
We address how stem cell biology is exploited to maintain intestinal cancers by developing new functional approaches to assaying stem cells in situ. After validation in normal intestine, these end-points are applied to assess stem-like cells in cancers where they can be used to determine the efficacy of therapies. Renewing tissues and many cancers are maintained by a small number of long-lived stem cells. Most models of stem cell organisation take account of their longevity and the fact that they self-renew, and also assume that they are stable populations carrying unique identifying characteristics. For decades the assays used to test different cell populations for their ‘stemness’ have appeared consistent with such deterministic models. These assays commonly challenge the ability of cells, separated into discrete populations based on the expression of cell surface antigens, to undergo growth when cultured or engrafted. Cells that are able to support long-term growth are viewed as being synonymous with stem cells.

However, this interpretation of stem cell organisation now seems too simplistic. For example: cell fate is likely determined by small changes in the expression of regulatory transcription factors in the context of transcriptional networks; the cell surface signatures of stem cells may not be as stable over time as previously thought; the success of stem cell engraftment may be partly determined by properties of the recipient rather than the transplanted cells (Chang et al, Nature 2008; 453: 544; Quintana et al, Nature 2008; 456: 593). Stem cell biology may be driven by stochastic switching between different states in response to variations in the balance of signals coming from complex transcriptional networks. In accordance with this view we have recently demonstrated, by following the dynamics of clonal growth in situ, that intestinal stem cell turnover is a constant and rapid stochastic process that follows a pattern of neutral drift (Lopez-Garcia et al, Science 2010; 330: 822).

Given the above our approach is pragmatic: to identify novel ways of assaying stem cells in situ with respect to the functional end-points that are integral to their biology.

What is the multi-potentiality of stem-like cells in intestinal cancers?

Our long-term objective is to determine the repertoire of differentiation options available to cancer stem cells, how this differs from normal stem cells, and thereby identify unique opportunities for therapies. To measure potentiality we are exploiting the known differences between cell types in the timing of DNA replication during the cell cycle. Genes associated with maintaining pluripotency are replicated early in S-phase, while those associated with neural lineages are replicated late in S-phase (Azuara et al, Nat Cell Biol 2006; 8: 532). The pattern of replication timing for key transcription factors has been described as a barcode of potentiality, indicative of the accessibility of the chromatin for subsequent expression. We are attempting to devise such a barcode for intestinal stem cells to identify changes in potentiality during carcinogenesis. To date we have shown reproducible differences in replication timing between different loci. For example, the neural transcription factor Mash1 is replicated late, while the transcription factor Ngn3, expressed in the intestine, is replicated early. We aim to increase genomic coverage by amplification to generate a comprehensive characterisation of replication timing. The effect of deleting the APC tumour suppressor gene on replication timing patterns is also being determined — deleting this gene also results in dramatic changes in cell type (loss of secretory cell lineages) and differentiation.

Role of quiescent stem cells

Label retaining cells, identified by their ability to sequester and retain label, have long been thought to be synonymous with quiescent stem cells. Using inducible expression of nuclear-localised fluorescent protein (Histone H2B-YFP) we have identified a population of crypt-base cells that appear to divide either very slowly or to be quiescent. Conventional views of stem cell organisation would place these cells as potential...
long-lived cells acting at the apex of a proliferative hierarchy. However, such an interpretation is not compatible with the dynamics that we have documented: rapid stem cell turnover with neutral drift. It now appears that these cells are committed to become secretory Paneth cells and do not normally contribute to stem cell maintenance. However, they can do so following injury illustrating that they can be recalled to the stem cell compartment. Importantly similar quiescent secretory cells are found in tumours and are also clonogenic under regenerative conditions (Figure 1).

Cancer models and tumour progression
At a molecular level the development of intestinal cancers is well characterised, with the most common genetic changes incorporated into a paradigm of progression for colorectal cancers in which loss of APC is a central early event (as described by Bert Vogelstein’s lab at Johns Hopkins University). Despite this it has been shown that many other gene specific mutations can also be associated with the disease (Sjoblom et al., Science 2006; 314: 268). APC has been deleted in animal models by a variety of strategies that usually lead to the development of benign adenomas. Introduction of additional mutational events in candidate genes has only been partly successful in creating the full carcinomatous (cancer-like) disease. Our ability to induce deletion of APC in the intestinal epithelium lends itself to the investigation of the nature of other gene mutations that might interact with APC and contribute to the formation of malignant disease. Therefore, as an alternative unbiased approach to identifying such genes we recently described using our Cre models to mobilise a Sleeping-Beauty activated transposable element in mice predisposed to intestinal tumorigenesis by virtue of APC deficiency (Collier et al, Nature 2005; 436: 272). Cloning and sequencing of the insertion sites in tumours allows affected genes to be identified (as common insertion sites or CISs) and associated with tumour pathology.

Analysis of these insertion sites identifies hundreds of gene that are mutated in multiple tumours (March et al, Nat Genet 2011; 43: 1202). In trying to determine the significance of this observation we noted that: (1) tumours are very oligoclonal and arise against a background of a low rate of insertional mutation, presumably due to the process of Darwinian selection; (2) known oncogenic pathways are repeatedly mutated with insertions found in most tumours in one or more of the TGFβ superfamily, p53 or K-ras pathways; (3) no insertions were uniquely associated with subsets of tumours identified by pathological or other features but that certain genes were over-represented (e.g. in the case of tumours with abnormal Paneth cell differentiation); (4) some CISs co-occurred with a higher frequency than would be expected by chance. These observation suggest that tumours are repeatedly mutated in respect of many modifiers that are associated with different tumour contexts.

In moving forward from the interpretation that there may be many more genes involved in tumorigenesis we are trying to develop a first pass screen based on Next Generation Sequencing that determines the clonal representation of specific gene mutations in different tumour models.

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LNCaP prostate cancer cell stained by immunofluorescence for B10015 (green), phosphatidic acid phosphatase (red) and the nucleus (blue). Image provided by Jonathan Kay (Neal laboratory).
The CI’s Core Facilities provide state-of-the-art services and equipment to support the cutting-edge research of the Institute, as well as working towards applying new technologies to cancer research. Each facility has a team of scientific staff who provide scientific support, advice, and training for all CI researchers and students in the use of their facility’s particular speciality, as well as keeping fully up-to-date on developing technologies.
We provide a data analysis and statistics consulting service to CI scientists and develop software and analysis pipelines to support high-throughput technologies.

High-throughput sequencing applications have continued to be a major theme for the group over the past year, leading to the development of analysis pipelines for ChIP-seq experiments to explore binding of proteins to DNA and for the re-sequencing of cancer genomes to identify mutations implicated in the disease. These pipelines are computationally expensive but are structured into workflows where tasks are distributed across many processors on the high-performance compute cluster. We have also gained experience in analysis methods for RNA-seq experiments in which gene transcripts are read using Illumina HiSeq sequencing instruments.

In other work, we have supported projects involving a range of experimental techniques including microarrays for analysis of single nucleotide polymorphisms (SNPs) and copy number variation, tissue microarrays for determining protein expression across multiple cancer types, and various imaging, flow cytometry and proteomics applications.

A further aspect of the Core’s work is the development of bioinformatics infrastructure to support automated data processing, which this year required a significant refactoring to support the increasing volumes of data produced, and the deployment of analysis tools and databases, particularly the Galaxy system that enables researchers to carry out analyses on their data and the Core to deploy analysis and visualization methods developed in-house.

We continue to work closely with our colleagues in the Genomics Core supporting the sequencing and microarray services and have been running weekly project review/experimental design meetings and statistics clinics. We ran training courses on differential binding analysis for ChIP-seq experiments, gene expression analysis using microarrays, introductory statistics, and a new course on experimental design.

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Circos plot showing chromosomal rearrangements and copy number gains and losses from whole genome sequencing of an oesophageal tumour sample.
The BRU facility within the CI has the ability to offer both a state-of-the-art animal facility and a variety of associated services to Cancer Research UK and its collaborators.

Isolation and Containment
The isolation and containment suite was primarily set up to allow animals with certain named pathogens to be housed and imaged within the CI, using facilities such as our Xenogen, MRI multi-proton microscope and PET/SPECT scanners. Animals that have been exposed to CL2 products will also be housed within this area, allowing work with potentially hazardous materials to be contained.

We also currently have four isolators. This will ensure that shipments are kept isolated from other shipments that may have the potential to pass on pathogens as yet unseen in the colony. It also ensures that they are segregated from the main colonies in the CI. From these we will have the potential to re-derive mice into the barrier should the goal be to establish a breeding colony within the CI.

A dedicated husbandry team ensures all husbandry needs are met and are also able to carry out specialist technical and licensed tasks on any researcher’s behalf.

Import/export programme
The animal model service at the CI has the responsibility of arranging the importation and exportation of whole animals, tissues and/or embryos and sperm to/from any external collaborators and/or commercial establishment located either nationally or internationally. As part of the service we carry out the following:

- Source particular strains
- Identify and apply for required government licences
- Complete import and excise paperwork
- Find the most suitable shipping agents
- Arrange transportation
- Liaise with both the shipping agents and the external collaborators.

Transgenic service
Our dedicated Transgenic team offer a variety of bespoke transgenic services. These include the cryopreservation of embryonic and sperm cells, the derivation of new mouse ES cell lines, siRNA transgenesis or embryo aggregation, embryo or oocyte collection and transgenic advice.

Regulatory compliance advisory service
The use of animals for scientific procedures is controlled by the Animals (Scientific Procedures) Act 1986. Three licences are required to be in place before any regulated procedures can take place. These are a personal licence (PIL), a project licence (PPL) and a certificate of designation (Cert Des). A regulated procedure under the Act is described as any experimental procedure applied to a protected animal that may cause pain, suffering, distress or lasting harm. This also includes procedures such as breeding. The regulatory compliance group offers a wide range of licensing tools and up to date advice designed to ensure that all local and national requirements have been appropriately addressed both at the beginning and throughout the duration of research projects.
We provide up-to-date expertise, training and troubleshooting in all aspects of cell and tissue culture, to maintain a consistent high standard throughout the Institute. The facility is used extensively by most CI research groups, and this year there has been a significant increase in the number of human tissue samples received, both for general research, and clinical trials use which must also comply with MHRA guidelines. We attend conferences, training courses and seminars on relevant aspects of cell culture and bio-banking which helps us to maintain a cutting-edge facility. We host an annual cell culture workshop where a panel of experts on mammalian cell culture offer help and advice to our scientists.

Cell culture
We provide basic cell culture training for all CI scientists, a comprehensive weekly mycoplasma testing service, a batch testing service for serum and other cell culture media components, and quality controlled bulk culture of research cell lines, including mouse embryonic fibroblasts (MEFs). We also offer a monthly routine human cell line authentication service using multiplex PCR and short tandem repeat (STR) profiling, which includes a mouse specific primer pair to detect any mouse cell contamination of human cell lines. These tests are important to confirm integrity of data and are becoming a requirement for publication in many leading journals, therefore we have seen an increase in demand.

We support three Essen BioScience IncuCyte™ instruments which are compact, automated imaging platforms designed to provide kinetic, non-invasive live cell imaging. The instruments are located in a 5% CO₂ incubator and acquire high definition phase contrast and fluorescent images of live cells in vitro in cell culture. Custom image processing software calculates a variety of metrics, such as cell proliferation, invasion and migration assays, growth curves and optimisation of cell based assays and cell culture media components. These instruments are very popular with all in continuous use.

The Human Tissue Act
Our staff advise on, monitor and control the import, use, storage and disposal of human tissue samples for research, to ensure full compliance with the Human Tissue Act (2004) and the Human Tissue Authority (HTA) Codes of Practice, a statutory requirement for all research involving human tissue samples. We advise on how to request human tissue samples from the Addenbrooke’s Hospital tissue bank and other sources, and how to obtain local research ethics committee approval for new research projects involving the use of human tissues.

Future developments
We have recently evaluated the latest IncuCyte™ instrument and other high-content imaging platforms with an interest in imaging 3D events such as measuring spheroid growth, cell invasion and angiogenesis. We are also investigating the possibility of operating one of our IncuCyte™ instruments in hypoxic conditions, which more closely mimics the in vivo conditions within a tumour. We are examining the feasibility of offering some of our current services to the wider Cambridge cancer research community.

Our service allows simple access to storage, tracking and risk management of tissue samples, cell lines and any other biological samples, including human tissue samples, in accordance with current legislation.
The Equipment Park provides CI scientists with access to a range of state-of-the-art equipment and specialised technologies.

Our lab offers technical/scientific advice, troubleshooting support and appropriate training for all the facility’s equipment. We also routinely test the capabilities of our equipment, optimise current or new techniques for our instruments and horizon scan to maximise the quality of data generated and to provide the best possible advice to CI scientists.

Protein gel electrophoresis
We provide access to a wide range of gel electrophoresis equipment for analysis of protein samples. We have the capability for both 1- and 2-dimensional separation of proteins including 2D Fluorescence Difference In-Gel Electrophoresis (2D-DiGE). Together with our range of digital camera and scanner imaging systems, we can digitise images which improves accuracy of quantification, saves time and reduces costs. This year we have optimised the technique of Western blotting with very low numbers of cells to support a number of groups wanting to analyse protein expression in flow-sorted cells.

Biosensor
The Biacore T100 measures molecular interactions in real-time. It provides label-free measurements of the affinity and kinetics of interactions, as well as the thermodynamic properties underlying association and dissociation rates. This instrument has proved pivotal in a number of research projects this year carried out by the Murphy laboratory, investigating the kinetics of protein-protein interactions. This year we have focused on optimising a new technique for the Biacore in collaboration with the Proteomics Core Facility, to capture and then identify novel binding partners for molecules in a situation where one binding partner is not known.

Plate readers and spectrophotometers
The Equipment Park provides access to three high specification plate readers. The Tecan Infinite M200 is used extensively by most research groups at the CI for absorbance, fluorescence and/or luminescence assay work, and we also house a BioTek Clarity, a dedicated luminescence plate reader. A third plate reader, a BMG PHERAstar FS, allows users to perform higher-end assays including time-resolved fluorescence or fluorescence polarisation and also increases sample throughput with its automated plate stacker. This year we have optimised the use of this plate reader for high-throughput analysis of nucleic acid quantification using Qubit assay kits, in line with the requirements of the Genomics core facility. A fourth UV-visible cuvette spectrophotometer, the Cecil Super Aquarius 9500, is particularly suited to quantification of low-concentration samples.

Imaging systems
Five imaging systems are available that produce digital images from a wide range of different samples. The Typhoon Trio produces images of radioactive, visible fluorescent or chemiluminescent samples while the Li-Cor Odyssey images fluorescence specifically in the infrared region. Both systems are used routinely for Western blotting and cell-based assays. The ImageScanner III is a high-resolution flatbed scanner for imaging non-fluorescent samples. We also have a high resolution camera system, the Syngene Dyversity, capable of capturing both fluorescent and chemiluminescent images. Dedicated analysis software packages can accurately quantify protein/DNA bands or spots captured by any of these imaging systems. This year we have also introduced a new dedicated colony counter to support the work of a number of groups in the Institute required to image spheroids.

Molecular biology applications
The Equipment Park houses an 8-channel NanoDrop as well as a Qubit for quantification of small volume nucleic acid (and protein) samples and has the capability for both standard and real-time PCR. We also have a pulsed-field gel electrophoresis system, CHEF III, for separation of large DNA molecules and an E-Gel iBase for fast separation of DNA and RNA.
The Flow Cytometry core facility provides state-of-the-art flow cytometric instrumentation, technical expertise, training, and software analysis in a collaborative environment. Our mission is to develop cytometric technologies that will best assist CI researchers in finding answers for the treatment, prevention, and understanding of cancer.

**Services**
Our lab offers a full range of educational and cytometric services that includes immunophenotyping, cell cycle analysis, translocation and co-localisation of cell activation markers, chromatin density, and apoptotic and necrotic analysis. In addition we are capable of performing cell sorting for researchers so that they can isolate cell populations needed for further studies.

Users are offered an array of educational programs in the theory, anatomy, applications and science of flow cytometry. Additional workshops are offered on data analysis using all of our software programs and on practical applications of current protocols in cytometry. We also collaborate with other scientists in the Cambridge Cancer Centre on our specialised equipment.

**Equipment**

**FACS Aria SORP (BD Biosciences)** – The Aria is a high-speed sorter. It is equipped with five lasers: a UV, 407nm, 445nm, 488nm, and 633nm. Our optical configuration allows us to see three UV, six violet, three indigo, six blue and three red parameters.

**LSR II (BD Biosciences)** – The LSR II is an analytical bench top flow cytometer. It is comprised of four lasers: a UV, a violet (407nm), a blue (488 nm) and a red (633 nm). Our optical configurations allow users to see two UV, six violet, seven blue and three red fluorescent parameters.

**FACS Caliburs (BD Biosciences)** – These flow cytometers are routinely used for phenotyping (to look at antigen, cytokine, or GFP expression), cell cycle analysis, and apoptosis studies. They are equipped with 488nm and 635nm lasers that allow users six parameter analysis.

**ImageStream (Amnis)** – The powerful combination of quantitative image analysis and flow cytometry in a single platform creates exceptional new experimental capabilities. 405nm, 488nm and 635nm lasers for four colour/six parameter analysis as well as EDF capability for FISH analysis are available.

**Influx Cell Sorter (BD Biosciences)** – This high speed cell sorter is contained within a biosafety cabinet to enable the isolation of cell populations from human tissue. It has four lasers at 405nm, 488nm, 561nm, 640nm and is equipped with 12 fluorescence detectors.

**RoboSep (Stem Cell Technologies)** – This magnetic bead separator unit has customisable programs allowing positive or negative selection of virtually any cell type from any species. Up to four samples can be processed simultaneously.

**Vi-CELL (Beckman Coulter)** – The Vi-CELL automates the widely accepted trypan blue cell exclusion method, with video imaging of the flow-through cell, to obtain results in minutes. The software conforms to key regulatory requirements with its electronic signature capability, audit trail, secure user sign on and user level permissions for clinical or preclinical studies.
The Genomics core facility allows researchers at the CI access to state-of-the-art DNA and RNA analysis instruments, methods and applications.

The tools in Genomics help researchers to understand the cancer genome, unravel the genetic causes of cancer and develop new methods for diagnosis, treatment, etc. Cancer genomics has been revolutionised by next-generation sequencing (NGS) technology and the people and instruments in the Genomics core help CI scientists answer their research questions. Microarray technology is still being used for copy-number and differential gene expression, and the core offers access to these and other genomic technologies as required by scientists at the CI.

In the last year it has become possible to sequence the entire genome of a cancer patient using NGS. It is likely to be several years before this technical possibility becomes a clinical tool, however we can now sequence a human genome in around five days and perform unbiased genome-wide experiments to see what the underlying sequence differences are in cancer genomes.

**Illumina NGS:** We make extensive use of the Illumina NGS instruments (Figure 1) and the CI has worked with this technology in both the Genomics and Bioinformatics core facilities for over six years, and is a centre of excellence. Having the capability to access new systems like this puts the CI at the forefront of genomic research. However, the technology continues to develop and Shankar Balasubramanian’s group recently published a novel method for the detection of 5hmC (Booth et al, *Science* 2012; 336: 934).

The genomics core was involved in many research projects last year but two stand out. The METABRIC project (Curtis et al, *Nature* 2012; 486: 346) from the Caldas group has changed our understanding about the complexity of breast cancer and identified 10 distinct sub-groups; this information will ultimately help in the treatment of patients. The core processed 1500 samples from tissue to nucleic acids, negotiated contracts for the copy number analysis and completed nearly 3000 gene expression arrays for the project. We also worked with the Rosenfeld group on their groundbreaking research into the use of circulating tumour DNA in a patients blood as a means to identify and monitor cancer (Forshew et al, *Sci Transl Med* 2012; 4: 136ra68). The core helped bring the Fluidigm technology into the Institute and worked with the Rosenfeld group on their methods and sequencing.

**Microarray:** Arrays continue to be used by many other groups at the CI although we are moving differential gene expression analysis to RNA-seq. We worked with the Neal group on their prostate cancer work demonstrating an *in vivo* restricted set of AR-regulated genes (Sharma et al, *Cancer Cell* 2013; 23: 35).

An important component of the Genomics core facility is our staff. The technologies we use are complicated and we undertake projects for the Institute’s research groups as well as training individuals to use Genomics core equipment. We also offer support and access to other genomics platforms including: Real-time PCR for lower throughput gene expression and copy number analysis; Pyrosequencing to look at methylation of DNA; Agilent Bioanalyser instruments to quality control RNA and DNA; and Qiagen robotics for nucleic acid extraction. We have recently installed a new system from Fluidigm that allows us to run very high throughput real-time PCR projects and also to amplify regions of the genome for targeted resequencing using NGS. This work is being used to develop new diagnostic tests for cancer.

*Publications listed on page 77*
The Histopathology/ISH core facility offers a variety of histological techniques, immunohistochemistry, in situ hybridisation, laser capture microdissection as well as automatic slide digitisation and analysis to CI scientists.

Histology
The facility processes, embeds and sections human and animal tissues or cell lines into frozen or paraffin formats and stains these with the standard H&E or special stains, as needed by the researcher to complement their work. During the past year, we added vibratome sectioning into our portfolio, which allows thick sectioning of tissue, from 50–250 µm, to gain better resolution of cellular interactions. We have also added further special stains, specifically Luxol Fast Blue/Crystal Fast Violet for Myelin fibres and a modification of the Masson Trichrome for better digital quantitation of collagen fibres (Figure 1).

Immunohistochemistry (IHC)
Through the course of the year, we have continued to work with our scientists to validate new antibodies onto the BondMax automated immunohistochemistry stainer, adding a further nine antibodies through this process and rejecting 10 antibodies as not specific and reliable. We have completed the work-up of the TUNEL method onto the BondMax, an application that allows for the detection of breaks in DNA, particularly where there is cell death and apoptosis. We have also completed validation of three dual immunohistochemistry protocols (Figure 2) as well as developing a combined immunohistochemistry/in situ hybridisation method. Finally, we have received approval for the replacement of two of our BondMax systems, with upgraded research instruments, the Bond III and the Bond Rx, which will allow us to further develop and increase the sensitivity of our methods onto the automated platforms in the next year.

In situ hybridisation (ISH)
This year we evaluated and modified the Panomics ISH kits from Affymetrix in order to replace the standard radioactive ISH with a robust non-radioactive method, and have validated 16 separate probes on this system. In addition, we have continued to run our standard miRNA staining method and fluorescence ISH (FISH) for the Y chromosome and human/mouse centromeric regions. In 2013 we aim to fully automate the Panomics method onto the Bond Rx as well as extend the dual staining immunohistochemistry/in situ hybridisation protocols that we offer.

Digitisation and analysis
We have digitised 35,000 slides through our three scanners (Leica Ariol SL50, Aperio XT, Zeiss Mirax), approximately a third of the slides that we produce. We continue to work with the increasing number of researchers who are using image analysis in order to accurately analyse their data. Finally, we have been part of the Citizen Science project, which uses “crowd sourcing” methods to automate the visual scoring of ER, PR and HER2 for breast cancer (www.clicktocure.net). Crowd sourcing involves training members of the public in tissue recognition and scoring and comparing the results to automated image analysis that has already been performed as well as to manual pathologist scoring. Our aim in 2013 is to further improve the image analysis methods available to our scientists.

Publications listed on page 77
The Light Microscopy Facility provides the CI with state-of-the-art light microscopy and develops new imaging modes.

The facility specialises in: advanced live-cell imaging using wide-field and spinning disc imaging systems; confocal scanning light microscopy; nonlinear imaging techniques such as multi-photon, second harmonic, CARS and fluorescence life-time imaging (FLIM); in vivo imaging at high-resolution; quantitative high-content image acquisition and analysis.

In 2012, two new staff members joined the facility: Alexander Schreiner (SSO, previously Watt laboratory) and Imran Patel (Postdoc, CCC pump priming funded).

The LaVision TriMScope system, equipped with an additional optical parametric oscillator, provides fs-pulsed MP-excitation ranging from 690 nm to 1600 nm. The TriMScope is a very sensitive and rapid multi-photon scanning system. In 2012, we added CARS imaging to the fluorescence and SHG imaging capacity of the MP-system in collaboration with Sumeet Mahajan (Department of Physics, Cambridge, now University of Southampton). This project has been awarded a CCC pump-priming grant, which allows us to explore a variety of projects with CI researchers.

The CompuCyte iCys imaging cytometer is the most popular quantitative high-content imaging and analysis system in the facility. Current applications include measuring ligand uptake, apoptosis, tumour vasculature and drug distribution and DNA damage in cancer cells, and tissue microarrays. We published a paper on characterization of surface FAS with quantitative morphological analysis using quantitative imaging cytometry (Ireland-Zecchini et al., *Curr Protoc Cytometry* 2012; 59: 12.25.1).

We have upgraded the live cell imaging systems with the newest sCMOS camera, an eNeo (Andor Inc.), which is simultaneously delivering ultra-low noise, fast frame rates (100 fps at full frame), wide dynamic range, high resolution and a large field of view for fast live-cell imaging.

We are also constantly monitoring new developments in imaging techniques. We have tested and applied high resolution imaging (OMX) in collaboration with the Wellcome Trust Gurdon Institute and CI colleagues (Sir et al, *Nat Genet* 2011, 43; 1147; Narita et al, *Science* 2011; 332: 966). We have successfully published the application of a supercontinuum white-light laser for interferometric biological imaging (Chiu et al, *J Microscopy* 2012; 246: 153).

An EMBO-funded annual course in Plymouth (www.mba.ac.uk/embo-course/) has become a centre for training and discussion in advanced optical microscope methods, attracting leading lecturers, manufacturers and students from the whole of Europe, including students from the CI. We continue to contribute to the MONAPHOT Erasmus Course (coordinator: Prof Zyss, ENS Cachan, France) by hosting masters students.

**Research and Development**

Current projects include the following:

(1) We are using second harmonic imaging based on a scattered signal, e.g. to demonstrate the formation of vessels from endothelial cells as well as the extracellular matrix in tumours, and cell behaviour in collagen matrices. We are working with Carola Schoenlieb (DAMTP, Cambridge) to develop image analysis tools to quantify tumour/stroma ratios in cancer.

(2) We are developing CARS imaging as a method for label-free apoptosis studies with Sumeet Mahajan and Christian Steuwe (Southampton and Cambridge).

**Publications listed on page 77**

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*joined in 2012 †left in 2012*
We work with the remit to provide bioanalytical, pharmacokinetic and pharmacodynamic support for the Institute.

Pharmacokinetics (PK) is the study of what the body does to drugs. It is the mathematical study and description of the absorption, distribution, metabolism and excretion processes used by the body when a drug is administered. In order to obtain good PK data, bioanalysis forms an integral part of the science as we need to quantify how much of the drug is in the body over a period of time. To facilitate this we have two liquid chromatography-mass spectrometry systems (LC-MS/MS) within the facility (Figure 1). These state-of-the-art systems enable us to detect very low levels of drugs in a variety of biological matrices such as blood, plasma, tumour and cell cultures. We have developed a range of bioanalytical assays to support various research groups ranging from small dicarboxylic acids to steroids. Our main highlight of 2012 was the development of a fast, robust and sensitive assay for the simultaneous detection and quantification of deoxycytidine, hydroxymethyl-deoxycytidine, methyl-deoxycytidine, deoxyadenosine and deoxyguanosine in DNA digests. In addition several bioanalytical assays have been validated to support clinical trial studies with further potential clinical trial support planned for 2013.

In terms of instrumentation, the Core PK/PD Facility has just purchased the latest instrument in mass spectrometry triple quadrupole technology: an ABSciex 6500 and this will replace the aging AB4000 in January 2013. This new instrument offers an increase in sensitivity thus providing better assays for the quantification of low abundance molecules such as hmdC (hydroxymethyl-deoxycytidine) and use of smaller sample volumes such as tissue obtained from needle biopsies.

Pharmacodynamics (PD) is the study of what the drug does to the body (i.e. its effect). By relating PD effects to PK parameters, the PK/PD relationship can be determined. To this end, a variety of PD assays (e.g. biomarker assays) were established to support several clinical trials. We are looking to expand our portfolio for 2013.

As we are working with clinical samples the facility is compliant to the MHRA guidelines entitled ‘GCP in the Clinical Laboratory’.

In addition to the analysis of PK samples we can also offer advice on the design of PK and efficacy studies.
Pre-clinical imaging is a collaborative facility that manages a wide range of imaging machines for the CI.

**Optical**

IVIS 200 and IVIS lumina imaging systems (Caliper Lifesciences) are available for whole-animal in vivo photonic imaging, including sensitive and relatively high-throughput in vivo bioluminescence imaging. Typical scans take less than one minute and up to five subjects can be imaged at a time.

**MRI**

We have two Varian MRI systems; a 9.4T with higher sensitivity, and a 7T whose smaller susceptibility effects make it more suitable for techniques such as echo-planar imaging. Both perform $^1$H MRI and multi-nuclear MRS, assisted by integrated monitoring, gating, heating and anaesthesia. We have produced DCE-MRI data for vascular characterisation of autochthonous pancreatic tumours (Tuveson laboratory). We have implemented improved $^1$H MRS methods that minimise chemical shift artefacts and we are developing quantitative MT-MRI and motion-insensitive DW-MRI methods for abdominal tumours, which are subject to respiratory and cardiac motion. Our Hypersense system is now routinely producing a wide range of hyperpolarised substrates for high-sensitivity $^{13}$C in vivo tumour metabolism studies, which employ fast spectroscopic imaging sequences.

**Metabolomics**

The facility is based on a Bruker 600MHz NMR instrument. High resolution $^1$H, $^{13}$C and $^{31}$P NMR studies are performed routinely on solution samples. An HRMAS $^1$H and $^{31}$P NMR probe allows biochemical analysis of intact ex vivo clinical and preclinical biopsies. Ongoing collaborations include studies on cellular senescence (Narita laboratory) and $^{19}$F NMR of anticancer drug metabolites (Tuveson laboratory). With the Tavaré laboratory we are developing metabolite correlation methods to interpret the biochemical data.

**Ultrasound**

Our ultrasound imaging provision includes two Vevo 2100 systems and one Vevo 770 system (Visualsonics). These image to as low as 30 micron resolution, providing excellent anatomical and soft tissue structural detail instantaneously and in real-time, and also permit rapid 3D imaging and dynamic vascular imaging with power Doppler and non-linear contrast.
The Proteomics core facility focuses on the systematic study of proteins, particularly their structures, interactions and expression levels. The facility is equipped with state-of-the-art instrumentation for CI researchers requiring access to proteomics technology and expertise.

The Proteomics core facility provides help in designing experimental strategies and implements and validates previously developed proteomic workflows to profile proteins from diverse biological samples. We also aim to modify or develop entirely new methods and assays when warranted. In addition, we have bioinformatics support for data management and analysis as well as software development.

The facility has already been well equipped with state-of-the-art analytical instrumentation for proteomic studies, including the latest Orbitrap mass spectrometers: an LTQ Velos Orbitrap (Thermo), which has been configured to a Dionex Ultimate 3000 RSLC nanoHPLC system (Figure 1), and a newly acquired Q Exactive Orbitrap. The mass spectrometers are supported by off-line chromatography platforms: two Dionex Ultimate 3000 capHPLC systems for multidimensional chromatography at the protein and peptide level. These are supported by 1D and 2D gel electrophoresis systems as well as a GE Healthcare Typhoon Trio+ imager available in the equipment park run by Jane Gray. Data analysis is supported by an array of bioinformatics and statistical analysis tools.

Specific methods and areas of interest include:

- Protein profiling of complex biological samples, e.g. serum, tissue, cell extracts
  - Profiling by nanoLC/MS
  - Multidimensional protein/peptide fractionation by capLC and/or geLC.

- Targeted protein identification by nanoLC/MS/MS
  - Coomassie and silver stained gel bands of purified proteins
  - In solution digestion of purified proteins.

- Identification of protein and peptide modifications
  - Phosphorylation sites
  - Protein modifications such as acetylation and methylation,
  - Coomassie stain only, purified proteins.

- Relative quantitation by nanoLC/MS/MS
  - SILAC - stable isotope labeling of amino acids in cell culture
  - iTRAQ - an isobaric peptide tagging system.

Publications listed on page 77
A confocal microscopy image of IMR90 (human diploid fibroblast) cells expressing oncogenic H-Ras stained for tubulin (red), trans-Golgi network (pink) and lysosomes (green). DNA was counterstained using DAPI (blue). Image provided by Masako Narita (Narita laboratory).
Laser scanning cytometer mosaic image of pancreatic tumour section labelled with DAPI (nuclei - blue), Hypoxyprobe (hypoxic areas - green) and lectin-647 (blood vessels - red) showing areas of hypoxia within the tumour and surrounding tissue. Image taken by Heather Zecchini (Light Microscopy core facility), sample provided by Mike Jacobetz (Tuveson laboratory).
INSTITUTE INFORMATION
Shankar Balasubramanian

Chemical biology of nucleic acids laboratory

Primary research papers

Bell NM, Kenyon JC, Balasubramanian S and Lever AM. Comparative structural effects of HIV-1 Gag and NC proteins in binding to and unwinding the viral RNA packaging signal. Biochemistry. 2012; 51: 3162-9


Tumbarello DA, Temple J and Brenton JD. Beta3 integrin modulates transforming growth factor beta induced (TGFβI) function and paclitaxel response in ovarian cancer cells. Mol Cancer. 2012; 11: 36


Kevin Brindle

Primary research papers


Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med. 2012; 4: 136ra68

Kuo CH, Xian J, Brenton JD, Franz K and Sivinah E. Complex stiffness gradient substrates for studying mechanotactic cell migration. Adv Mater. 2012; 24: 6059-64


Tumbarello DA, Temple J and Brenton JD. Beta3 integrin modulates transforming growth factor beta induced (TGFβI) function and paclitaxel response in ovarian cancer cells. Mol Cancer. 2012; 11: 36


Kevin Brindle

Primary research papers


Carlos Caldas (page 18)

Breast cancer functional genomics laboratory

Primary research papers


**Other publications**


**Jason Carroll** (page 20)

Nuclear receptor transcription laboratory

**Primary research papers**


Theodorou V, Stark R, Menon S and Carroll JS. GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. *Genome Res.* 2013; 23: 12-22

**Other publications**


Robinson JL and Carroll JS. FOXA1 is a key mediator of hormonal response in breast and prostate cancer. *Front Endocrinol (Lausanne).* 2012; 3: 68

**Douglas Fearon** (page 22)

Cancer and immunology laboratory

**Primary research papers**


Fanni Gergely (page 24)
Centrosomes, microtubules and cancer laboratory

Primary research papers

John Griffiths (page 26)
Magnetic resonance imaging and spectroscopy laboratory

Primary research papers

Other publications

Duncan Jodrell (page 28)
Pharmacology and drug development group

Primary research papers

Other publications
Cook N, Jodrell DI and Tuveson DA. Predictive

Florian Markowetz (page 30)  
Computational biology laboratory

**Primary research papers**

**Cao** A, Wang X, Fletcher MN, Meyer KB and Markowetz F. Reder: R/Bioconductor package for representing modular structures, nested networks and multiple levels of hierarchical associations. *Genome Biol.* 2012; 13: R29


**Other publications**


Adele Murrell (page 34)  
Epigenetics and imprinting laboratory

**Primary research papers**


Masashi Narita (page 36)  
Mechanisms of cellular senescence laboratory

**Primary research papers**


Other publications


Chandra T and Narita M. High-order chromatin structure and the epigenome in SAHFs. Nucleus. 2012; 4: Epub 13 Dec


Perez-Mancera PA, Guerra C, Barbadic M and Tuveson DA. What we have learned about pancreatic cancer from mouse models. Gastroenterology. 2012; 142: 1079-92

David Neal (page 38)

Prostate research laboratory

Primary research papers


Gu德mundsson J, Sulem P, Gudbjartsson DF, Masson G, Agrnasson BA, Benediktsson KR, ... Neal DE, Kiemeney LA, Thorsteinsson U, Rafnar T and Stefansson K. A study based on whole-genome sequencing yields a rare variant at 8q24


Zuccolo L, Lewis SJ, Donovan JL, Hamdy FC, Neal DE and Smith GD. Alcohol consumption and PSA-detected prostate cancer risk - a case-control nested in the PROTECt study. *Int J Cancer.* 2012; Epub 1 Oct

Other publications

Robinson JL and Carroll JS. FoxA1 is a key mediator of hormonal response in breast and prostate cancer. *Front Endocrinol (Lausanne).* 2012; 3: 68

Duncan Odom (page 40)

Regulatory systems biology laboratory

Primary research papers


Faure AJ, Schmidt D, Watt S, Schwalie PC, Wilson MD, Xu H, Ramsay RG, Odom DT and Flicek P. Cohesin regulates tissue-specific expression by stabilising highly occupied cis-
regulatory modules. Genome Res. 2012; 22: 2163-75


Other publications


Bruce Ponder (page 42)

Genetic susceptibility to cancer laboratory

Primary research papers


Nitzan Rosenfeld (page 44)

Molecular and computational diagnostics laboratory

Primary research papers
**John Stingl** (page 46)
Mammary stem cell biology laboratory

**Primary research papers**


**Other publications**

**Simon Tavaré** (page 48)
Statistics and computational biology laboratory

**Primary research papers**


**Bioinformatics** (page 54)
Matthew Eldridge

**Primary research papers**


Other publications


Our researchers produced or updated the following software packages this year.

BADGER v 1.0
A tool to identify sample mixups in large expression and genotyping studies.
Maintainers: Andy Lynch, Mark Dunning (Tavaré lab, Bioinformatics)
r-forge.r-project.org/projects/badger/

beadarray v 2.8.1
Quality assessment and low-level analysis for Illumina BeadArray data
Maintainers: Mark Dunning, Mike Smith, Jonathan Cairns, Andy Lynch, Matt Ritchie (Bioinformatics and Tavaré lab)

BeadArrayUseCases v 1.0.9
Examples and tutorials for running Illumina expression analyses with BioConductor.
Maintainers: Mark Dunning, Wei Shi, Andy Lynch, Mike Smith, Matt Ritchie (Bioinformatics and Tavaré lab)
www.bioconductor.org/packages/release/data/experiment/html/BeadArrayUseCases.html

CRImage
Image analysis of histopathologically stained tumour slides
Maintainer: Yinyin Yuan (Markowetz lab)

DiffBind
Differential binding analysis of ChIP-seq peak data
Maintainer: Rory Stark (Bioinformatics)

DIRECT v 1.0
A tool to cluster replicated gene expression data from a time-course experiment or from multiple experimental conditions.
Maintainer: Audrey Fu (Tavaré lab)
cran.r-project.org/web/packages/DIRECT/index.html

IDATreader v 0.1.1
A tool to read encoded Illumina IDAT files
Maintainer: Mike Smith (Tavaré lab)
www.compbio.group.cam.ac.uk/software/idatreader

Mulder2012
Maintainer: Xin Wang (Markowetz lab)
bioconductor.org/packages/release/data/experiment/html/Mulder2012.html

PANR
Posterior association networks from functional genomics data.
Maintainer: Xin Wang (Markowetz lab)
www.bioconductor.org/packages/release/bioc/html/PANR.html

RedeR
Network Data Integration, Analysis, and Visualization in a Box.
Maintainer: Mauro Castro (Markowetz lab)
bioconductor.org/packages/release/bioc/html/RedeR.html

Rcade v 1.0.0
A tool for integrating ChIP-seq data with expression data to identify genes targeted by a transcription factor of interest.
Maintainer: Jonathan Cairns (Tavaré lab)
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Kerstin Timm (Brindle laboratory)
Caldas laboratory

Merck
Tuveson laboratory

Microsoft Research
Brenton laboratory

Movember
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Aileen Marshall (Odom laboratory)
Christine Parkinson (Brenton laboratory)
Naomi Sharma (Neal laboratory)
Maxine Tran (Neal laboratory)
Ponder laboratory

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Tuveson laboratory

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Pedro Correa de Sampaio (Murphy laboratory)
José Sandoval (Caldas laboratory)

Prostate Cancer UK
Ajoeb Baridi (Stingl and Neal laboratories)
Hayley Whitaker (Neal laboratory)
Neal laboratory

Royal Society University Research Fellowship
Fanni Gergely

Science and Technology Facilities Council
Brenton laboratory

The Leukemia and Lymphoma Society, USA
Piotr Dzien (Brindle laboratory)

Uehara Memorial Foundation
Hiro Fujiwara (Watt laboratory)
Mahito Sadaie (Narita laboratory)

Wellcome Trust Senior Investigator Award
Shankar Balasubramanian
SEMINARS AND CONFERENCES

The CRUK CI hosts a number of seminar series, covering basic to translational aspects of cancer research, and quantitative biology.

CRUK CI Seminars in Cancer
We welcomed the following speakers to present in our international seminar series, CRUK CI Seminars in Cancer:

Erwin Wagner, CNIO Madrid
Inflammatory skin disease and cancer: functions of AP-1 (Fos/Jun) in mice and humans

David Pellman, Dana-Farber Cancer Institute, Harvard Medical School
Chromosome segregation and genome stability

Ihor Lemischka, Mount Sinai Medical Centre, New York
Pluripotency, Etc.

John Dick, University of Toronto
Towards unification of genetic and hierarchy models of tumor heterogeneity

Kári Stefánsson, deCODE Genetics, Iceland
Common and rare variants in the sequence of the human genome that affect the risk of cancers and other diseases of man

Thea Tlsty, University of California, San Francisco
Cellular conversations that control cell fate

Maria Blasco, CNIO, Madrid
The role of telomeres and telomerase in stem cell biology, cancer and ageing

Hans Clevers, Hubrecht Institute, The Netherlands
Lgr5 stem cells in self-renewal and cancer

Mark J. Ratain, Director, Center for Personalized Therapeutics, University of Chicago
Challenges in building a genomic prescribing system

Frank McCormick, UCSF Helen Diller Family Comprehensive Cancer Center
The Ras pathway in cancer therapy

Details of all CI seminar series – CRUK CI Seminars in Cancer, Cambridge Oncology Seminars, Lectures in Cancer Biology and CRUK CI Seminars in Quantitative Biology – can be found on www.cruk.cam.ac.uk/seminars-and-conferences

Cambridge Cancer Centre Annual Symposium, 22 June
See page 84 for full information.

Institute Retreat, 11–12 October
This year’s retreat was once again held at the Institute. We continued with the popular talks for non-scientists series, which was well attended. We had three types of scientific talk: research talks, recent results presentations and clinical case presentations. These provided an excellent opportunity to review work in progress, assess successfully published results, and gain insight into the clinical applications of the CI’s research. The team building activity of creating a geodesic dome from newspaper and tape produced structures with varying degrees of structural integrity.
CI Symposium, Unanswered Questions in Cancer Sequencing, 2–3 November
This year we welcomed a distinguished line-up of international speakers to speak about and discuss questions in cancer sequencing, in a programme put together by the scientific organising committee of Shankar Balasubramanian, Carlos Caldas, Jason Carroll and Simon Tavaré.

Session 1: Cancer genome sequencing
Mike Stratton (Chair), Wellcome Trust Sanger Institute
The evolution of the cancer genome
Sean Grimmond, University of Queensland
Cohort and personalized cancer genome studies of pancreatic cancer
Seishi Ogawa, University of Tokyo
Genetic analysis of myeloid neoplasms in childhood
Binay Panda, Ganit Labs, India
Landscape of genetic changes in oral tongue squamous cell carcinoma (selected talk from submitted abstracts)
Carlos López-Otín, Universidad de Oviedo, Spain
The genomic landscape of chronic lymphocytic leukemia
Levi Garraway, Dana-Farber Cancer Institute
Models of tumor evolution: biological and therapeutic implications

Session 2: Challenges facing the computational biology of cancer sequencing
Ewan Birney (Chair), European Bioinformatics Institute
ENCODE: Understanding our genome
Jan Korbel, European Molecular Biology Laboratory
Mechanisms of genomic structural variation in germline and cancer
Elaine Mardis, The Genome Institute, St Louis
Genomic heterogeneity in cancer cells
HyunChul Jung, National Cancer Center, Korea
Systematic survey of cancer-associated somatic SNPs (selected talk from submitted abstracts)
Elliott Margulies, Illumina, Inc
Delivering clinically relevant genome information
Richard Durbin, Wellcome Trust Sanger Institute
Human genetic variation: from population sequencing to cellular function

Session 3: The impact of cancer sequencing on medicine
Keith Peters (Chair), GlaxoSmithKline
Lori Friedman, Genentech, Inc.
Overcoming resistance to targeted therapies in breast cancer

Peter Lichter, German Cancer Research Centre
Sequencing of paediatric brain tumours: from molecular profiles to clinical translation
Marloes Hoogstraat, University Medical Center Utrecht, The Netherlands
The Dutch Center for Personalized Cancer Treatment: moving towards implementation of next-gen into clinical decision-making (selected talk from submitted abstracts)
Nitzan Rosenfeld, Cancer Research UK Cambridge Institute
Circulating tumour DNA as a non-invasive tool for cancer diagnostics and research
Bruce Ponder, Cancer Research UK Cambridge Institute
How can we use knowledge of genetic variation?

Session 4: Functional genomics of cancer
Carlos Caldas (Chair), Cancer Research UK Cambridge Institute
Functional genomics of cancer: perturbation experiments in the lab and in the clinic
René Bernards, The Netherlands Cancer Institute
Finding effective combination therapies for cancer through functional genetics
Dale Porter, Novartis
Using genomic data coupled with preclinical response data to guide clinical development of IAP antagonists
Rebecca McIntyre, Wellcome Trust Sanger Institute
High-throughput functional validation of candidate colorectal cancer genes (selected talk from submitted abstracts)
Jason Carroll, Cancer Research UK Cambridge Institute
Understanding oestrogen receptor transcription in breast cancer

2013 events
24–25 October: CI Retreat
8–9 November: CI Annual International Symposium – Unanswered Questions in Cancer Metabolism
The Cambridge Cancer Centre (CCC) is a formal partnership between Cancer Research UK, the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust. The vision of the CCC is to build strong links across disciplines from the laboratory to the clinic.

The CCC was launched in 2007 as a virtual framework for interaction of the 142 research groups working in cancer across Cambridge. This year the Centre has made significant strides in setting out its 5- and 10-year strategies, identifying its research activities and resources across Cambridge, analysing the current cancer services and the interface between those and clinical research, and streamlining funding. We also started, and have nearly completed, the accreditation and designation of the Centre by the Organisation of European Cancer Institutes. The external peer review visit was in July and we hope to hear the outcome in the next few months.

The CCC has an expanding cohort of PhD students and clinical fellows. There are currently eight PhD students and five clinical fellows, with another four PhD students and two clinical fellows joining them in 2013. The students and fellows are undertaking their research in departments and Institutes across Cambridge. The Centre also supports four PhD students on the Cambridge PhD Training Programme in Chemical Biology and Molecular Medicine.

The Centre awards a number of pump-priming grants every year to fund novel and interdisciplinary projects between at least two different University departments or Institutes. Four projects were funded in 2012 and two projects were funded at the start of 2013. These six projects are truly collaborative, involving 10 different departments/institutes between them: the Departments of Applied Mathematics and Theoretical Physics, Biochemistry, Chemistry, Physics and Zoology; the Cancer Research UK Cambridge Institute; MRC Laboratory of Molecular Biology; Babraham Institute; MRC Cancer Cell Unit and Wellcome Trust/Cancer Research UK Gurdon Institute.

The 6th annual CCC Symposium took place in June with around 300 delegates from a wide range of University departments, institutes and biotech companies in Cambridge. The Keynote Lecture was given by Frederic de Sauvage (Genentech Inc.), who gave a very interesting talk on targeting the hedgehog pathway with small molecule antagonists (in this case Vismodegib, GDC-0449) in patients with basal cell carcinoma and medulloblastoma. The talks from Cambridge-based researchers spanned a range of disciplines: Phil Jones (MRC Cancer Cell Unit) – oesophageal progenitor cells and carcinogenesis; Brian Huntly (Cambridge Institute for Medical Research) – targeting novel proteins in leukaemia; Peter Campbell (Wellcome Trust Sanger Institute) – next-generation sequencing and somatic mutations in cancer; Tony Green (Cambridge Institute for Medical Research) – cancer-associated chromosome deletions; Ben Simons (Department of Physics/Gurdon Institute) – cellular hierarchy in skin tumour growth; KJ Patel (MRC Laboratory of Molecular Biology) – factors that induce endogenous DNA damage; David Klenerman (Department of Chemistry) – new biological insights from single molecule studies; Nitzan Rosenfeld (CRUK CI) – circulating mutant DNA as a non-invasive diagnostic tool for cancer; and Doug Winton (CRUK CI) – heterogeneity in intestinal progenitors. A successful poster session took place over lunch, and the day ended with the well-known barbecue! Unfortunately this year, for the first time in the six years of the Symposium, it rained, and so it was an indoor barbecue!
This year we opened the doors of our Institute to our Cancer Research UK (CRUK) fundraisers and supporters, schoolchildren and the general public. We held an Open Day one afternoon in May, which proved to be a big success. The labs and core facilities were all open for our guests to walk around freely and engage with the researchers and support staff. There was also a programme of short talks on subjects ranging from ovarian cancer to developing new drug combinations throughout the event, which greatly appealed to our guests.

This year we again took part in the Cambridge Science Festival, which is the UK’s largest free science festival and attracts around 35,000 visitors over a two week period. We also again hosted the East Regional Final of the Institute of Ideas Debating Matters competition and a few members of staff were involved in judging the debates. The competition is held nationally for sixth-form students to debate current issues in science, politics, the arts and other topics.

We had a wonderful afternoon event in November to thank Phil Purdy and his 50 supporters. Phil ascended Everest in May and raised a staggering £125,000 for oesophageal and pancreatic cancer research. The ‘thank you’ event involved lab tours, talks from Sir Bruce Ponder (Director, CI), Dr Rebecca Fitzgerald (MRC Cancer Cell Unit) and Ed Roberts (Postdoc, Fearon group, CI), and a film of Phil’s Everest ascent. One of the highlights of the event was the Everest cake, complete with a marzipan Phil, which researcher Dr Hayley Whitaker (Head, Biomarkers Initiative) had created for the event.

Our researchers regularly give talks at local primary and secondary schools, as well as at CRUK fundraising and supporter events (e.g. Race for Life, Relay for Life and Stand Up to Cancer). We also host visits of the Institute for groups of CRUK fundraisers and supporters; around 30 per year. It is always very inspiring to meet the people who give so much of their time and go to great lengths to support CRUK and our research.

CI staff are actively involved in public engagement and fundraising events.
The graduate student body in the Cambridge Institute is composed of PhD students, MB/PhD students and clinical research training fellows. The details of the graduate training programme are co-determined by Cancer Research UK, the Institute, the University Department with which a student is affiliated, and the University of Cambridge.

Support and Mentoring
Each student has a supervisor who is a group leader and is also assigned a second supervisor who acts as a mentor and provides additional support. If a problem or question arises that is not directly related to their project, Ann Kaminski (the Head of Scientific Administration) acts as the first point of contact for any student with a query or difficulty. All student matters in the Institute are overseen by the Studentships and Fellowships Committee, chaired by John Griffiths. This committee has the well-being of our students at heart, while ensuring that they are fulfilling the requirements of the University of Cambridge to obtain their degree.

The Graduate Programme
Soon after their arrival, all of our new graduate students join the University graduate intake to attend the compulsory introductory safety and induction courses organised by the University, followed by similar courses specific to the Institute. Ann Kaminski hosts a reception for all students, GRADSOC committee members and group leaders to provide an early opportunity for students to meet some of the Institute personnel who will help them over the years to come.

All graduate students are required to attend a series of around 30 lectures in cancer biology. This ensures that all our students, despite their diverse backgrounds and knowledge bases acquire a good grounding in cancer biology. The lectures are given by specialists in their fields and they provide the students with a comprehensive overview of cancer biology, ranging from basic cell biology through to cancer diagnosis and treatment. This excellent and unique resource is available to all members of the University and is widely attended. The students are also introduced to the core facilities managers and their teams to learn about the services available to them that may be of great use during their study period. The students also attend courses specific to the demands of their projects, some of which are organised within the Institute and others by the wider University.
After two months of study, all first year students give short talks to all members of the Institute to explain the nature of their projects. This allows them to engage with a wider range of research staff, who advise and encourage. In accordance with University regulations, all graduate students studying Biological Sciences in Cambridge are not at first registered for PhD studies and must qualify for registration by successfully completing a first year report followed by a viva. Two examiners assess each student, and the report and comment on the student’s suitability to continue for PhD study.

Our second, third and fourth year PhD students give research talks as part of the Institute Lunchtime Seminar series, attended by all Institute staff. In addition, students complete a written report towards the end of their second year which summarises their work to date and also forms the basis for discussions regarding further work. Our graduate students all follow the three year graduate programme supported by the University of Cambridge; a further year is available if necessary to complete their thesis, which must be submitted within four years.

Like their colleagues in London, our students are encouraged to attend numerous courses planned to hone their transferable skills. These courses range from advice on how to make scientific posters to the Cancer Research UK-organised Graduate Students Public Engagement with Science and Technology (GRADPEST) course.

The Graduate Society (GRADSOC)
The graduate students have organised themselves into a very active society which organises many varied activities, including monthly journal clubs and a wide variety of social events including movie nights, a cake club, punting and the occasional wine-tasting. The society also arrange meetings with visiting speakers and have a Christmas dinner with an invited speaker — this year’s speaker was The Right Honourable Professor The Lord Winston, expert in human fertility. The society has also introduced a highly effective mentoring scheme in which all first year students have two student mentors who are located in different parts of the building. This provides new students with recognisable friendly faces in other labs and also helps them to settle in much quicker.

Other Student Activities
Many of the students have attended conferences and workshops both in the UK and overseas, where they have presented their research findings either as a poster or as a talk.

What next?
Of the 15 students who submitted their theses in 2012, two have accepted positions in industry, six have become post-docs (in Brussels, Italy, Copenhagen, MRC-LMB, CRUK CI and University of Southern California), one has returned to medical school, two have returned to clinical practice and another has been accepted for study by the Graduate Medical programme in Cambridge.

Awards, Prizes and Achievements
Several of our students won prizes or were invited to speak at meetings this year:

Elke Van Oudenhove (Brenton laboratory) and Sarah Jurmeister (Neal laboratory) were selected by The Compass Group to receive research funding from the proceeds of their annual ball held on 01 July. Elke and Sarah attended the ball at which they gave talks. The ball raised in excess of £80,000, which was donated to CRUK.

Simon Buczacki (Winton laboratory) won The Kevin Burnand Prize, Society for Academic and Research Surgery, Annual Meeting, Nottingham, UK, and also the prize for Poster of Distinction, American Gastroenterological Association, Digestive Disease Week, San Diego, USA.

Muhammed Murtaza (Rosenfeld laboratory) won a prize under the Academy of Medical Sciences’ Clinical Research Champion Scheme for best oral presentation from a clinically qualified presenter at the School of Clinical Medicine’s Research day in June.

Richard Wells (Fearon laboratory) won a CORE/Dr Falk award for UK medics doing gastroenterological research which he collected at a BSG society dinner held in Liverpool in June.

Ana Tufegdžić-Vidaković (Caldas laboratory) won the best poster in the Epigenetics section of the CI Symposium and the second best poster in the School of Clinical Medicine Research Day.

Ajob Baridi (Stingl and Neal laboratories) was a member of the University of Cambridge team that won the Biotechnology Young Entrepreneurs Scheme (YES) competition run by the BBSRC and the University of Nottingham Institute for Enterprise and Innovation. The team’s hypothetical company Calvitium Solutions won the £1,000 prize and the team will travel to Houston, Texas in April to represent the UK in the International YES competition.
The administration team facilitates the smooth running of the Institute by providing infrastructure and support to the Director.

The team provides administrative support to group leaders and supports research activities through management of the laboratories and core facilities. The team also coordinates graduate student administration and laboratory finance, the Cambridge Cancer Centre (page 84) and outreach activities (page 85). In addition to laboratory management each of the group leaders has administrative support provided by one of the dedicated research administrators.

This year the whole team were heavily involved in the transfer of the Institute to the University of Cambridge. An impressive team effort saw the legal transfer take place on time: the signing of the transfer documents took place on 18 December with completion on 1 January. In the first few months of the year we will focus on learning new policies and procedures and completing non-essential transfer tasks.

Scientific Administration

Graduate student and summer student administration is overseen by Ann Kaminski (page 86). The team organises tenure reviews and mid-term reviews for the research groups, and reviews for the core facilities.

The scientific administration team is responsible for the running of symposia, seminars, chalk talks and committees that take place in the Institute, including providing full audio visual cover. The team also organise the CI symposium and the group leader and Institute retreats. We produce the Institute’s publications including the annual report, leaflets and posters for fundraising and public engagement activities, write for and edit the intranet and internet sites.

The team is also responsible for internal and external communications, coordination with the Cancer Research UK and University of Cambridge press offices, and is involved in the organisation of fundraising visits. For more details of public engagement and fundraising activities see page 85.

Human Resources

Human Resources (HR) work in partnership with the Institute to provide support and guidance in the areas of recruitment, personal and team development, pay and grading, employment law and staff wellbeing. The Institute has always enjoyed a mixed economy of staff from both Cancer Research UK and the University of Cambridge so collaboration has been essential to provide a seamless employment experience, allowing the Institute to focus on its research.

This year has seen the Institute bring these two groups together on a more formal basis as the Institute became part of the University of Cambridge. This involved close collaboration of HR from both CRUK and the University Clinical School to facilitate the smooth transfer of employment for CRUK staff based at the Institute. Throughout the process staff engaged with the consultation and its goals of increasing benefit to cancer research efforts in Cambridge.

In addition as predicted at the end of 2011, the Institute’s HR continued to interact with the wider Cambridge HR community in creating an environment conducive to high quality research.

Finance

The finance team:

- Help budget holders efficiently manage their budgets.
- Provide financial analysis to CRUK CI management to inform decision-making.
- Assist with the budget and business planning process for the CRUK CI.
- Assist with the acquisition and management of grants.
- Provide a link between the University/Clinical School finance departments and the CRUK CI.
- Help with ad hoc queries and concerns.

Laboratory Management

The Laboratory Management team form part of the Institute administration and continue to provide a vital role in underpinning the Institute’s ever-evolving research activities. The team assists the twenty research groups by maintaining the tissue culture rooms (Containment Levels 1 and 2) and communal equipment parks situated on the first and second floors as well as providing training for users on the apparatus within those rooms. The distribution of plastic consumables and specialist gases is organized by the team as well as...
the annual pipette clinics and chemical amnesties. The team was instrumental in the organisation and subsequent success of the Institute’s Open Day in May and is currently involved in the relocation of some expanding laboratories, which will continue into the early part of 2013.

Glasswash and Media
The Glasswash and Media Core Facility provides a high quality, centralised glass washing and sterile supplies service offering a range of basic solutions and liquid/solid media which are replenished in the laboratories on a daily basis. Specialised buffers or solutions are also available using the Request service. The team also manages the tissue culture media consignment stocks and supply centres as well as organising the sterilisation of class I genetically modified (GM) and containment level 2 waste.

Health and Safety
We are committed to the continuous improvement of the safety performance of the Institute. Our philosophy is to encourage staff to take ownership for their own safety, and thereby create a positive safety culture. As a Health and Safety team we help staff develop pragmatic safety solutions that safeguard their welfare, and also ensure regulatory compliance.

During 2012 we conducted safety inspections, and initiated a program of safety audits, with a view to ensuring that good safety standards are maintained in the laboratories. We continued to developed new policies, revised existing policies and delivered safety training. We also made preparations for the transition of the Institute from CRUK over to the University of Cambridge.

In 2013 we aim to align our safety policies and procedures with those of the University. We will continue to improve safety performance at the CI, and encourage managers to promote good safety performance within their departments. We will improve safety communications by having defined key safety contacts in each department. We will deliver safety training to meet the needs of the Institute and continue our inspection program.

Procurement
The CI procurement team is supported by the Purchasing Operations Team, which is based in London. Procurement helps departments accomplish their objectives by undertaking a range of activities that achieve value for money. We do this through good procurement practice and increased efficiency whilst mitigating operational, commercial and compliance risk to Cancer Research UK.

- We are working with key suppliers to set up central discounted price agreements, reducing the cost of consumable items.
- We are working with laboratory management teams in London and Cambridge to set up central service contracts.
- We are working across directorates on a number of proposals to implement new ways of buying goods and services into the organisation, enabling further efficiencies through increased automation of buying processes.
- We are playing our part in helping Cancer Research UK meet its environmental targets. We are integrating sustainability into our procurement process and are working with our suppliers to minimise waste and packaging and to support recycling schemes.

From January 2013 procurement will be managed by the University Finance Division.

Property Services
Property Services adopt a Total Workplace Management strategy, working towards integrating the provision of all services to the Institute. We are ambitious and progressive and we work alongside the end-users of these services to deliver and maintain a world-class facility, whilst reducing our environmental, economic and operational impact wherever possible.

The Property Services team provide three core services: maintenance, facilities and security. Our key objective is to ensure the smooth, safe and efficient running of Institute operations, in order to support the CI’s research efforts.

We ensure that the building is clean, well maintained and secure for staff and visitors. Property Services provide and administer many services including but not limited to the following services: electrical maintenance, cleaning, car parking, environmental and climate control systems, post room, recycling, catering, mechanical engineering, physical and electronic security, access control, patrolling and monitoring of various alarms and equipment, deliveries and reception, decorating and repairs, furniture, energy management and carbon reduction, waste removal and disposal, reprographics and photocopying, catering and hospitality, stationery.
The CI hosts a large number of seminars and events for staff and external visitors. Property Services manages the organisation and hospitality requirements for these events.

The team liaises with other organisations on the Cambridge Biomedical Campus and beyond to ensure that the CI is kept up to date with developments within the sector, it also contributes to the site's environmental and sustainability objectives.

In 2012 the Property Services team were awarded the British Building Maintenance Awards for its efforts within the industry sector. The team is also a member of the BIFM, CIBSE and EI, amongst other national associations.

**IT and Scientific Computing**

Researchers at the Institute depend on information technology for nearly every aspect of their work, from the collection and analysis of experimental data to the development of new theoretical techniques. The IT and Scientific Computing team provides technology and expertise to support these needs.

The department has ten staff with a variety of technical and programming skills to provide a helpdesk, systems administration, database support, application development, and design and build services for new information systems. They are drawn from a variety of scientific computing and IT backgrounds with experience in industry and academic research organisations.

The Institute’s scientists (and their laboratory devices) use a variety of Apple, Windows and Linux desktops and laptops to support their work, with Voice Over IP (VOIP) phone systems and 1Gb network connectivity to labs and offices. All users have access to backed-up network file systems to store and share their data.

Around 20% of the researchers at the Institute have roles which are primarily mathematical or computational. Many of the technologies in use (sequencing, proteomics, imaging, etc.) depend on large scale computational data analysis, with tens of terabytes of data being generated each week. To support this the department provides a blade-based computer cluster, parallel file system storage for high throughput analysis, mirrored disk storage for working data, archive storage for long-term retention of raw experimental data and final analysis results, and servers for databases and web applications.

During 2012 the team implemented major changes in the way services were provided to scientists, as the Institute became more independent in IT terms and made increasing use of the University’s infrastructure. Infrastructure improvements included the commissioning of local firewalls and 10Gb core networking; the archive was expanded from 480TB to 720TB. New services included an Institute Exchange email service, intranet web site, internet web site, Sharepoint document repository, guest wireless network and new core wireless network. Network access was moved from the CRUK wide area network to the JANET academic network and the CRUK telephones were replaced by Cambridge University VOIP extensions.

The team also secured funding from the European Commission to work with the Cambridge semiconductor company ARM, investigating the potential of novel low-energy computing technologies to support the Institute’s research.
The following CI students submitted theses in 2012:

**PhD**

Hamid Raza Ali, Caldas laboratory  
Molecular classification of breast cancer: histology-based assays and clinical significance

Hélène Bon, Neal laboratory  
A study of kinases at the interface between metabolic stress and cell cycle control in prostate cancer

Joana Borlido, Neal laboratory  
Exploring the relationship between clathrin and mitosis

Simon Buczacki, Winton laboratory  
The molecular and functional characterisation of intestinal label retaining cells

Pedro Correa De Sampaio, Murphy laboratory  
Using a novel 3-dimensional in vitro spheroid model to investigate new roles for stromal metalloproteinases in tumour angiogenesis

Mike Fletcher, Ponder laboratory  
Fibroblast Growth Factor Receptor 2-regulated gene expression in breast cancer

Raj Giraddi, Stingl laboratory  
Cell cycle kinetics of mammary stem and progenitor cells

Chris Hurley, Winton laboratory (with Anna Philpott, Hutchison-MRC Research Centre)  
Regulation of the transcription factor Neurogenin 3 by post-translational modification

Brett Kennedy, Brindle laboratory  
Probing tissue metabolism in a murine model of lymphoma with hyperpolarised ¹³C-labelled substrates

Sarah Kozar, Winton laboratory  
Determining the stem cell dynamics of intestinal tissues and cancer

Shen-Han Lee, Griffiths laboratory  
¹H magnetic resonance spectroscopic imaging of tumour extracellular pH: the role of carbonic anhydrase IX

Angelika Modelska, Caldas laboratory  
Mechanisms of translation dysregulation in breast cancer

**MPhil**

Anna Brown, Griffiths laboratory  
Novel MRI techniques for early detection of brain metastases

Paweł Schweiger, Watt laboratory  
The role of ΔNLEf1 in epidermal differentiation, lineage selection and cancer

Joo-Hee Sir, Gergely laboratory  
Characterisation of the autosomal recessive primary microcephaly complex, CEP63-CEP152 in the vertebrate centrosome

Andrea Sottoriva, Tavaré laboratory  
Spatial cell ancestral inference: determining in vivo cancer dynamics from patient molecular data
CONTACT DETAILS

Cancer Research UK Cambridge Institute
University of Cambridge
Li Ka Shing Centre
Robinson Way
Cambridge CB2 0RE

Telephone: +44 1223 769500
www.cruk.cam.ac.uk

An electronic copy of this report is available on our website.

By road: from M11 junction 11, follow the signs to Addenbrooke’s Biomedical Campus, or follow the signs to Trumpington Park and Ride and take the Guided Bus.

By rail: take the train to Cambridge Station, then take the Guided Bus (Route A to Trumpington Park and Ride) or Stagecoach Citi 1, Citi 7 or Citi 8 bus to Addenbrooke’s Bus Station.

By air: the nearest airport to the CRUK CI is London Stansted Airport.

Cancer Research UK
Cancer Research UK is registered as a charity in England and Wales (1089464), Scotland (SC041666) and the Isle of Man (1103).
Registered as a company limited by guarantee in England and Wales number: 4325234
Registered address: Angel Building, 407 St. John Street, London EC1V 4AD

Telephone: +44 20 7242 0200
www.cruk.org
Cover image

Derivative work of a 3D reconstruction of z stacks showing DNA and β-catenin staining in a high-grade serous ovarian cancer tissue. The image was collected using the Leica TCS SP5 MP microscope. In this image, the DNA is pseudo-coloured in rainbow and β-catenin is red. Original image provided by Gayathri Chandrasekaran (Gergely laboratory).